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Animal and Plant Health Inspection Service

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BEHAVIORAL-PHYSIOLOGICAL EFFECTS OF RED PHOSPHORUS SMOKE INHALATION ON TWO WILDLIFE SPECIES

FINAL, TASK 2 REPORT

(Effective Smoke Concentration Range-finding Determinations)

PRINCIPAL INVESTIGATORS (Scientific)
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R. D. Thompson

April 1989

U.S. DEPARTMENT OF AGRICULTURE
Animal and Plant Health Inspection Service
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Effects of red phosphorus/butyl rubber (RP/BR) smoke on 2 wildlife species, prairie dogs and rock doves, were evaluated in laboratory range-finding experiments. Prairie dog groups showed no mortality within 30 days post exposure to 0.0, 2.0, 4.0 and 6.0 mg/l target concentrations of the smoke over 1 to 4, 80-min sessions. Rock doves exposed in groups to 0.0, 3.0, or 6.0 mg/l target concentrations over 1 to 4 sessions, however, showed 26 percent mortality within 8 days post exposure. Male rock doves were much more vulnerable to RP/BR smoke effects with 42 percent mortality in contrast to 6 percent in the females. Symptomatology assessments indicated: affected or lost vocalization capability in both species, abnormal body postures in rock doves, and increased respiratory congestion in prairie dogs post exposure. Body weight losses were severe and protracted in male rock doves. The 6.0 mg/l groups did not recover their losses in mean body weight during the 28-day observation period. Prairie dogs, ~~in contrast, only~~ showed a 1-day body weight loss and complete recovery to pre-exposure levels within 3 days post exposure. Both species showed significantly elevated water consumption levels on Days 10 through 28 after exposure. This measure was also directly affected by smoke concentration and number of exposure sessions. Neither species showed strong, consistent effects in gross

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necropsy and histopathology assessments. Only in a few rock doves exposed to the 6.0 mg/l level of smoke were there signs of excess mucus or exudate in the nasal passages and larynges. Selection of maximum RP/BR smoke concentration levels for future (Task 3) behavioral-physiological studies was based upon measured carbon monoxide levels, as well as, rock dove mortality. The 4.0 mg/l level administered over 2 daily, 80-min exposures was chosen with a predicted mortality of less than 10 percent in rock doves. Four daily, 80-min exposures at this level were predicted to cause no deaths in prairie dogs. A minimum smoke concentration was chosen to be 1.0 mg/l in order to minimize flame-out problems and to thus ensure temporal stability.

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EXECUTIVE SUMMARY

This project is designed to evaluate certain toxicological, behavioral and physiological effects of red phosphorus/butyl rubber (RP/BR) smoke on two wildlife species--black-tailed prairie dogs and rock doves. Project research has been divided into 3 tasks: Task 1--Inhalation Equipment Development/Ambient Carbon Monoxide (CO) Evaluation/Aerosol Distribution and Air Quality Study, Task 2--Effective Smoke Concentration Range-finding Determinations, and Task 3--RP/BR Effects upon Spontaneous Activity, Startle Response, Pulmonary Function, and Blood Chemistry of Prairie Dogs and Rock Doves.

This report describes the findings of Task 2 research. The general approach with both species involved 3 main areas of gross toxicological range-finding assessment of RP/BR aerosol effects: symptomatology/mortality, gross necropsy and histopathology. Mortality and symptomatology measures were used to characterize lethal effects (number of surviving animals post exposure, number of days until death) and sublethal effects (body posture, respiratory congestion, coat or plumage condition, vocalization effects, water consumption levels and body weights). Gross necropsy examinations were performed on all animals after 28 days of post-exposure observation. Surviving animals were euthanized with sodium pentobarbital injection and a team of USDA/APHIS veterinarians performed the necropsies examining the following organs for abnormalities: nasal passages, trachea, larynx, epiglottis, bronchi, lungs, heart, liver, spleen and kidneys. Abnormalities were recorded on standard forms for later analysis, and tissue sections of the first 6 listed organs plus the liver were preserved in formalin solution for later histological examination. The histopathology examinations were conducted at the National Veterinary Services Laboratory (USDA/APHIS).

Both RP/BR range-finding studies on each species used the same 3-phase paradigm: Pre-exposure, Exposure and Post-exposure. The Pre-exposure Phase was the 7 days prior to RP/BR exposure(s). Independent groups of animals (n = 6) then received 1 to 4 successive daily 80-min RP/BR-aerosol or filtered-air exposures during the Exposure Phase. The Post-exposure Phase was a 30-day observation/assessment period. Mortality was determined daily. Symptomatology data were collected continuously during the first 2 phases and on the first 7 days of the Post-exposure Phase. Thereafter, data were collected every third day through Day 28 post exposure.

Prairie dog groups were exposed to 2.0, 4.0 or 6.0 mg/l target concentrations of RP/BR aerosol or filtered-air (0.0 mg/l) over 1 to 4 successive sessions. Rock dove groups were exposed to 3.0 or 6.0 mg/l target concentrations of RP/BR-aerosol or filtered-air also over 1 to 4 successive sessions.

Mortality rates for the 2 species were vastly different within the RP/BR aerosol ranges tested. No prairie dogs died over the 30-day observation period post exposure, but 11:42 or 26 percent of RP/BR aerosol exposed rock doves died within 8 days post exposure. Ten of these deaths occurred in groups that received the 6.0 mg/l-aerosol target concentration, and a sex difference in mortality was also noted. The RP/BR smoke exposures were lethal to 42 percent of the males but only to 6 percent of the females.

Symptoms shown by both species post exposure were similar in part. Both species showed affected or lost vocalization. Prairie dogs given the 6.0 mg/l RP/BR-aerosol level also showed increased respiratory congestion. Rock doves displayed abnormal body postures in 6 of 7 RP/BR smoke exposure groups.

Body weight effects were most pronounced in rock dove groups. The mean body weights of males compared to females were more severely depressed post exposure. Male survivors in the 6.0 mg/l level groups did not recover to their pre-exposure body weights for the entire 28-day post-exposure period. In contrast, prairie dog groups only showed a 1-day loss in continuous body weight gain followed by recovery within 3 days of post exposure.

The water consumption measures indicated that, for both species, a greater number of exposures to RP/BR smoke led to more elevated consumption levels on Days 10 through 28 post exposure. Both species also showed significant Concentration x Session interaction effects. The effects, however, were opposite for prairie dog versus rock dove groups. Whereas the 6.0 mg/l smoke-exposed prairie dog groups showed the highest mean water consumption late in post exposure (Days 10-28), the 3.0 mg/l smoke-exposed rock dove groups showed higher mean water consumption levels compared to the 6.0 mg/l smoke-exposed doves for this period. This paradoxical species difference was explained in terms of unavailability of late post-exposure data due to high mortality rates in those rock dove groups receiving 6.0 mg/l smoke exposures.

Necropsy data and histopathology data analyses yielded no strong, consistent effects in either species. There were only a few increased incidences of excessive mucus or exudate in nasal passages and larynges of those rock dove groups exposed to 6.0 mg/l RP/BR smoke over 3 and 4 successive sessions.

Task 2 mortality results affirmed that both black-tailed prairie dogs and rock doves are more resistant to the lethal effects of RP/BR smoke than albino laboratory rats. The range-finding results, along with Task 1 data indicating potential high CO values (35 ppm) at the 6.0 mg/l smoke concentration level, led to our selection of 4.0 mg/l as the maximum target concentration to be used in Task 3 studies. Rock dove mortality at this level for 2 exposure sessions was predicted to be tolerable at less than 5 to 10 percent; prairie dog mortality at this level for 4 exposure sessions was predicted to be nil. The minimum target concentration for Task 3 was selected to be 1.0 mg/l in order to minimize RP/BR flame-outs and re-ignition problems, and to thus maintain high temporal homogeneity during animal exposures.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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R D Thompson   10/10/89
PI Signature   Date

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Veterinary Services personnel (APHIS/VS) were extremely cooperative in providing us the gross necropsy evaluations for this study. Appreciation is extended to Drs. Gary W. Church, Richard D. Brewster, Patricia L. White, L. J. Redder and Robert T. Tanaka. Dr. Gary W. Church and Dr. Albert J. Dale (APHIS/ADC) provided assistance and advice on animal quarantine, care, and euthanasia.

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Ken Crane, Stan Gaddis, Fritz Bush and Jeff Homan provided technical support throughout the Task 2 research. Ken Crane and Stan Gaddis conducted most of the RP/BR-aerosol exposure sessions including temperature, humidity, contaminant, particle size and phosphoric acid measurements. These technicians also assisted in all range-finding data collections with the prairie dogs and rock doves.

Jerry Rosencranz (Full Spectrum, Lakewood, CO) provided technical drawings of the inhalation and aerosol-sampling equipment.

Statistical consultation, data entry, and analyses were performed by Rick Engeman and Paige Groninger. Their perseverance in setup and analyses of the large data sets is greatly appreciated.

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I. INTRODUCTION

Red phosphorus butyl rubber (RP/BR) is the active agent in smoke grenades used to conceal troop movements by the U.S. Army (Burton, Clark, Miller, and Schirmer, 1982). Upon detonation, the grenades produce a dense white smoke consisting almost entirely of finely divided phosphoric and polyphosphoric acid (e.g., H_3PO_4, $H_4P_2O_7$) particles, along with trace amounts of carbon monoxide (CO). Potential health and environmental risks associated with RP/BR smoke are being assessed by the Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL).

Burton et al. (1982) evaluated the lethal effects of RP/BR combustion products over a range of concentrations extending from 1.5 to 8.5 mg/l in albino rats. Based on these data, we calculated the LC_{50} of RP/BR smoke to be 2.46 mg/l for rats given 5, 1-h daily exposure sessions. Post mortem examinations of the rats in this study characteristically showed laryngeal and epiglottal injury after exposure to high concentrations of the smoke (i.e., >5.0 mg/l). Multiple exposures to these concentrations also produced pulmonary congestion, edema and hemorrhage.

In a later study, Aranyi (1983b) exposed separate groups of albino rats to concentrations of RP/BR smoke products ranging from 1.56 to 3.05 mg/l for 5, 1-h daily sessions. Mortality ranged from 5% to 90%, and the LC_{50} value was estimated to be 2.32 mg/l RP/BR aerosol for this dosing procedure. Aranyi expected that inhalation toxicity would be related to pulmonary irritation, edema, scarring and, possibly, a lessened resistance to later infectious agents; however, none of the gross pathology or histopathology examinations confirmed these expectations.

Subsequent research (Aranyi, personal communication, 1984) indicated that RP/BR smoke can produce lung sclerosis *in vivo* at 1.2 mg/l with 40 h of total exposure in laboratory rats. Later, in tests for pulmonary bactericidal activity *in vitro*, rat lung tissues showed reduced resistance to an infectious agent at low, repeated 1-h inhalation exposures of approximately 0.5 mg/l. Extended RP/BR-smoke-exposure regimens involving this 0.5 mg/l concentration produced varying decrements in body weight gain that was more pronounced for male than for female rats.

In recent years, environmental concerns have been raised regarding the effects of repeated RP/BR-smoke exposures and other aerosol agents on plant and animal species living on or near military training installations. Van Voris et al. (1986) have demonstrated that 2- to 8-h exposures of 5 plant species (sagebrush, ponderosa pine, short-needle pine, blando brome and bushbean) at 200 to 5,600 mg/m^3 concentrations of RP/BR smoke produces a variety of toxic effects including leaf-tip burn, wilting, chlorosis, desiccation and dieback. To date, however, there have been no published reports on the effects of RP/BR smoke on wildlife, particularly dose-response evaluations. This lack of data led to the current work.

Black-tailed prairie dogs (*Cynomys ludovicianus*) and rock doves (*Columba livia*) were selected as representative wild mammalian and avian models to document potential toxicological, behavioral and physiological effects of

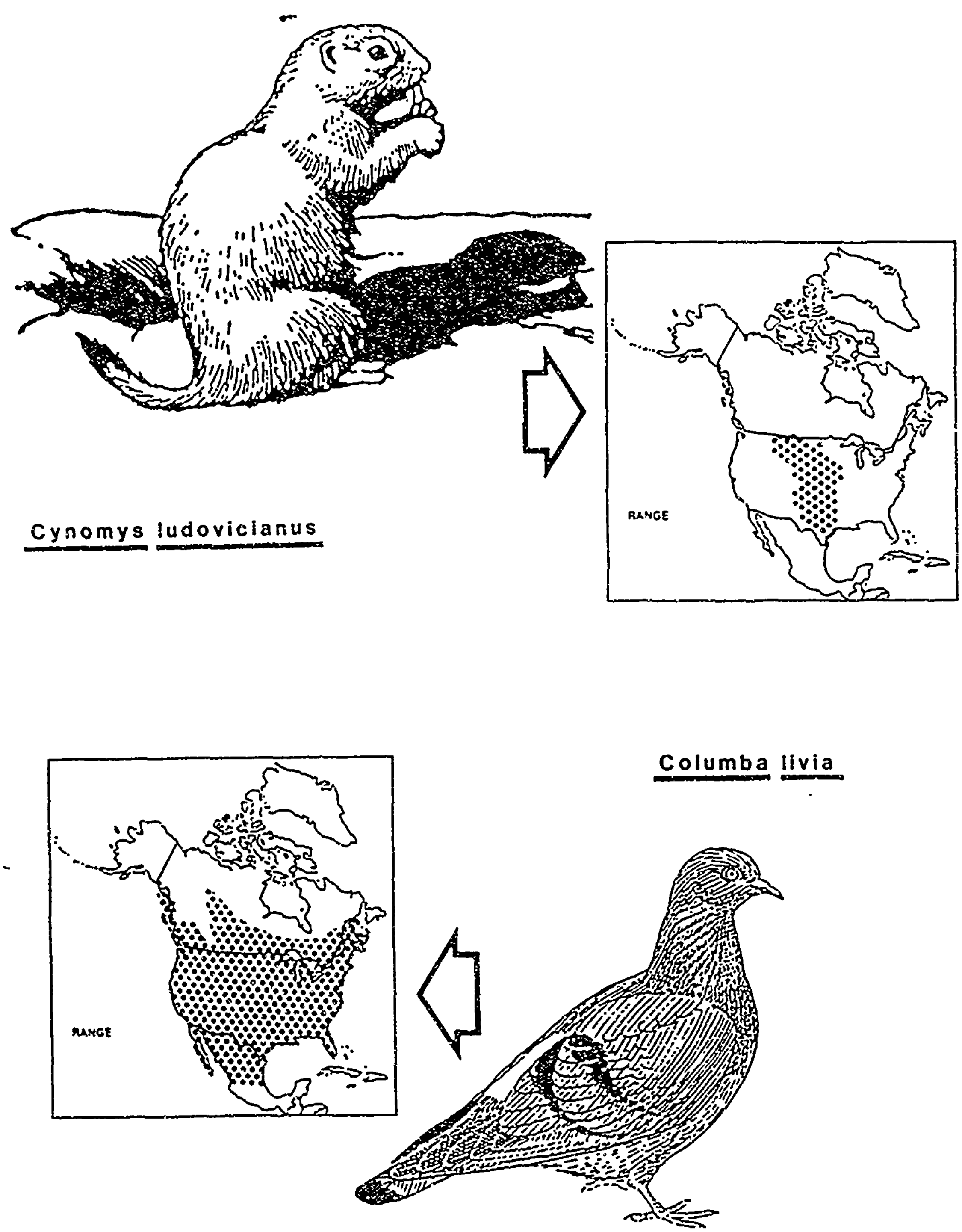

Figure 1. Drawings of a black-tailed prairie dog (Cynomys ludovicianus) and a rock dove (Columba livia), with distributional range maps for each species shown as inserts.

RP/BR-smoke exposure. As shown in Figure 1, both species share an extensive, overlapping range throughout the central United States. Data generated for these model species should provide general indications of potential RP/BR-smoke effects upon a variety of North American wildlife species (common or endangered). These data should prove useful to the U.S. Army, and to the public, in determining the environmental risks associated with frequent, repeated detonation of RP/BR grenades on military lands.

The main objective of Task 2 research is to determine gross toxicological effects of RP/BR smoke in concentration range-finding studies on these 2 wildlife species, prairie dogs and rock doves. Both species adapt well to confined laboratory conditions, and the behavioral and physiological effects of the RP/BR aerosol, if consistently identified, could be used to predict ecological effects upon certain wild mammal and bird populations living near or on U.S. Army training sites. The sublethal toxicological data (symptomatology, gross necropsy and histopathology) are also to be used for determining the levels of RP/BR smoke exposure in Task 3--consequences of RP/BR-aerosol inhalation upon pulmonary function, blood chemistry, spontaneous activity and startle response in these species.

II. DETERMINATION OF RP/BR AEROSOL TOXICITY EFFECTS

A. Approach

Three main sets of variables were selected to assess the toxic effects of RP/BR aerosol upon prairie dogs and rock doves: symptomatology/mortality, gross necropsy and histopathology. Symptomatology variables were used to characterize sublethal effects of RP/BR smoke upon such measures as body weight, water consumption, respiratory congestion and vocalization; whereas, lethal effects were quantified using such measures as number of animals surviving up to 28 days and number of days until death. After 28 days of post-exposure observation, surviving animals were euthanized with sodium pentobarbitol injection. Consulting veterinarians (APHIS, Veterinary Services, Denver, CO) then necropsied each animal reporting abnormalities observed in the respiratory tracts and lungs, heart, liver, spleen and kidney. Sections of organs were also removed from each animal at the time of necropsy, preserved in formalin solution, and sent to a pathology laboratory (APHIS, National Veterinary Services Laboratory, Ames, IA) for histological examination.

Conduct of the RP/BR-aerosol range-finding studies involved a standard 3-phase paradigm: Pre-exposure, Exposure and Post-exposure. The Pre-exposure Phase coincided with the 7 days prior to RP/BR-aerosol exposure. During the Exposure Phase, independent groups of animals (n = 6) received 1 to 4 successive daily 80-min RP/BR-aerosol or filtered-air exposures. The Post-exposure Phase involved an additional 30-day period for observation and assessment of RP/BR-caused effects in each group.

Throughout each Phase, mortality was determined daily. Symptomatology measurements were collected on each day of the Pre-exposure and Exposure Phases (i.e., immediately prior to RP/BR exposure) and on Days 1-7, 10, 13, 16, 19, 22, 25 and 28 of the Post-exposure Phase.

For purposes of data analyses, the mortality, gross necropsy, histopathology and certain symptomatology data (i.e., clinical observations) were treated descriptively (e.g., frequencies, percentages). The symptom categories of body weight and water consumption were analyzed using multi-factor analysis of variance (ANOVA) designs (Winer, 1971), with *post hoc* Duncan Multiple Range Tests used to assess significant effects found using ANOVA (Waller and Duncan, 1969).

The selection of appropriate RP/BR-smoke concentrations and exposures to be used in Task 3 were based on sublethal effects obtained from the aforementioned analyses. A relatively high and low sublethal treatment (i.e., RP/BR-smoke concentration x number of exposures) was sought for use with each species.

B. Methods and Materials

1. Animals

a. Black-tailed Prairie Dogs

Appendix A presents detailed descriptions of animal care procedures used for maintenance of prairie dogs during Task 2 studies.

All prairie dogs were captured at Buckley Air National Guard Base, Aurora, CO, during the month of February 1987. Approximately 95 percent of the 110 animals were captured by dispensing large volumes of soap and water into individual burrows (see Elias, Crier and Tietjen, 1974). As the burrows filled with soapy water, the prairie dogs exited and were hand-captured with a snare pole or gloves. Animals were towel-dried and immediately dusted with Purina Flea and Tick Powder to kill external parasites. The remainder of the prairie dogs were captured in wire mesh live traps (i.e., #203 Tomahawk Trap). After each day's capture session, animals were transported by vehicle to the DWRC quarantine facility.

Upon arrival at DWRC, the animals were re-dusted with Purina Flea and Tick Powder. Subsequently, each prairie dog was weighed (nearest g) and implanted with a subcutaneous transponder (Identification Devices, Inc., Boulder, CO) for individual identification (Fagerstone and Johns, 1987). The transponders were implanted using a 12-gauge needle at a site approximately 25 mm posterior to the animal's right ear in the dorsal neck region. Animal numbers could be "read" electronically by passing a hand-operated wand near the transponder. Animals were fed Purina Rabbit Checkers *ad libitum* and fresh cabbage 3 times per week during the 14-day quarantine period (April 15-29, 1987). After health checks (Veterinary Services, APHIS, USDA), animals were officially released from quarantine and were transported to Building 16 of the Denver Wildlife Research Center (DWRC).

A total of 72 animals (36 of each sex) was used for the range-finding studies. At Building 16 in Room 157S (see Insert Fig. 3) a 14-day acclimation-stabilization period was imposed on all test animals before the Pre-exposure Phase began. Animals were fed the same diet as during quarantine. During acclimation and throughout the range-finding studies, the prairie dogs were housed individually in either galvanized (51 x 27 x 38 cm) or in stainless steel cages (61 x 62.5 x 41 cm). The research rooms were temperature ($23^{\circ} \pm 2^{\circ}$ C) and light:dark (12:12 h) controlled throughout all acclimation and test phases.

b. Rock Doves

Appendix A offers a detailed description of the animal care procedures used with rock doves. A shipment of 122 rock doves was purchased from a local supplier on January 7, 1987. Birds were captured with cannon nets after a feeding site was prebaited with cracked corn (see Grubb, 1988); all birds were caught in the North Denver Area.

Upon receipt at DWRC, the birds were placed in wire mesh outdoor aviary cages (3.0 x 1.5 x 1.8 m). Up to 30 doves were held per cage, and the *ad libitum* maintenance diet was Purina Pigeon Checkers, cracked corn, grit, and water. After 13 weeks in this outdoor aviary, the doves were moved to an indoor quarantine facility. This was an 11.5-m-diameter steel Butler building, with heat and light provided.

At the time of transport to the quarantine facility, the birds were leg-banded with individual identification numbers, weighed and dusted with Purina Flea and Tick Powder. They were then checked for overt signs of poor health (Veterinary Services, APHIS, USDA) before being placed in one of 3 wire mesh communal aviaries (1.6 x 3.3 x 2.6 m; 2.0 x 6.6 x 2.6 m; 3.9 x 3.9 x 2.6 m). Fecal swabs were also collected from a sample of doves and analyzed for several avian diseases (e.g., Newcastle's Disease, Psittacosis). During quarantine, animals were maintained on Purina Pigeon Checkers and water *ad libitum* in this facility on a 12:12-h forward light:dark cycle for 29 days.

Upon completion of quarantine, the rock doves were moved to Building 16 (Room 160, see insert Fig. 3) and were allowed 14 days of acclimation-stabilization. A total of 48 doves (24 of each sex) was used during the range-finding experiments. Doves were held in this temperature ($23^{\circ} \pm 2^{\circ}$ C) and light:dark (12:12-h) controlled room in individual galvanized wire mesh cages (51 x 27 x 38 cm). The birds were fed the same diet as during quarantine.

2. Inhalation-exposure Systems

Two separate inhalation systems were used to expose animals to either doses of RP/BR aerosol or to equivalent durations of filtered air. The Modified RP/BR Extruder and Inhalation Chamber System was described in the Task 1 Report (Sterner et al., 1988). The Filtered-air Inhalation Chamber System was installed between 17 February and 1 May 1987 to provide for "control treatments" of independent groups of animals in Tasks 2 and 3.

Essentially, each System was constructed of identical materials and components, but each had independent closed-air supplies with separate air-filtration, air-humidification and air-movement equipment. Negative air pressure produced by individual ceiling vents (approx. 15-room air exchanges/h) within each system-housing room (i.e., Rooms 158 and 159, see insert Fig. 3) ensured against any unwanted exposure of animals in the animal-holding areas (i.e., Rooms 157S and 160, see insert Fig. 3) to potential traces of escaping RP/BR aerosol. Additionally, because exposures of animals to RP/BR aerosol always occurred prior to the conduct of the "filtered-air exposures" at a different time of day, the possibility of direct cross contamination between RP/BR-aerosol and Filtered-air Systems was negligible.

a. Modified RP/BR Extruder and Inhalation Chamber System

Figure 2 presents an illustration of the Modified RP/BR Extruder and Inhalation Chamber System. The insert of Figure 2 is a detailed drawing of the RP/BR extruder equipment.

A detailed description of the Modified RP/BR Extruder and Inhalation Chamber System is provided in Sterner et al. (1988). This System is similar to that described by Holmberg, Moneyhun, and Gayle (1985) and Aranyi (1983a, 1983b, 1984, and 1986). Key modifications to the original (ORNL) system involved the addition of certain air-filtration, air-humidification, temperature-regulation, and acid-resistant components (see Sterner et al., 1988). Essentially, results of aerosol uniformity and air quality tests showed the distribution of the within-chamber aerosol (i.e., aerosol mass, phosphoric acid, particle size) to be highly uniform, with acceptable volumes of respiratory gases (i.e., O_2 and CO_2) and with tolerable amounts of contaminant gases (i.e., CO, PH_3 and C_6H_{14}). Operation of the RP/BR-aerosol System can be summarized as involving 4 elements: the formulation of RP/BR product plus 3 subsystems of aerosol production and exposure (i.e., RP/BR extruder/generator, inhalation chamber, and air-movement/-condition/-filtration subsystems).

(1) Formulation of RP/BR product.--The RP/BR product was formulated by the staff of the Bio/Organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory (ORNL) in accordance with APHIS Interagency Agreements (IAGs) 87-74-01 and 34-WT-88 12-34-74-006 (IA). The mixture was

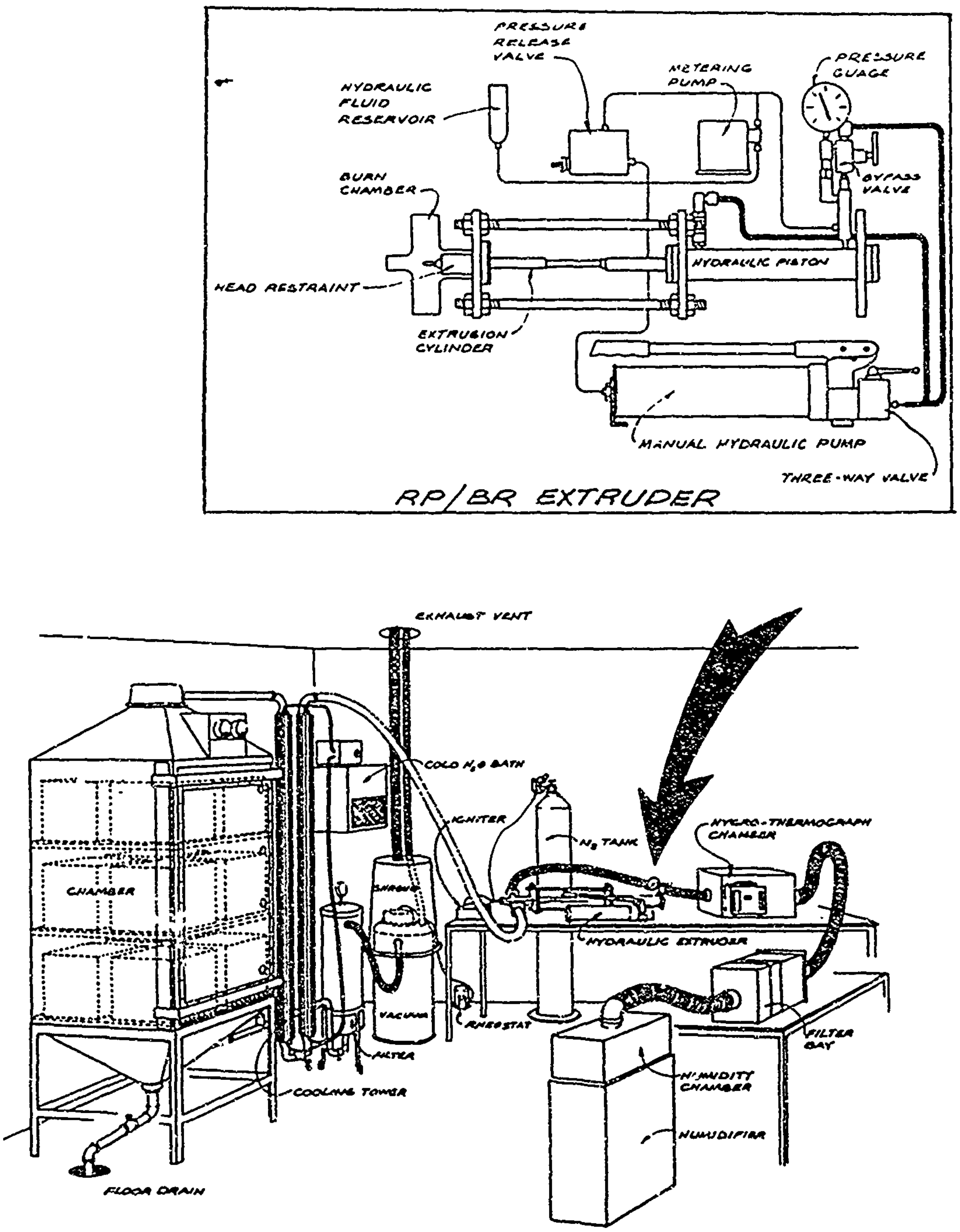

Figure 2. Technical illustration of the Modified RP/BR Extruder and Inhalation Chamber System. (Note.--Components of the System are scaled relative to the perspective, i.e., 2.54 cm equals 0.6 m, but the location of some components has been drawn to improve the visual display.) The insert shows a schematic diagram of the RP/BR extruder/generator subsystem--the equipment used to automatically extrude the softened RP/BR product into the glass burn chamber where it is ignited to generate aerosol in the intake airstream (from Holmberg, Moneyhun and Gayle, 1985).

formulated from 2.5 kg lots of a 95% RP (2.375 kg) and 5% BR (0.125 kg) product. Following mixing of the dry RP and BR substances, the product was placed in a vacuum desiccator and hexane was introduced until 7-8 percent (wt/wt) was absorbed. This "softened product" was then loaded into 11.45-cm sections of 1.91 cm i.d. stainless steel pipe (billets). Each of these "billets" contained approximately 40 g of pliable RP/BR material that was sealed with Teflon-lined steel caps to prevent drying. Billets were shipped to DWRC as needed, and only billets <3 months old were used to produce RP/BR aerosol for Task 2 studies.

(2) RP/BR extruder/generator subsystem.--The insert of Figure 2 presents a schematic drawing of the RP/BR extruder/generator subsystem. Operation requires loading the extrusion cylinder with the formulated RP/BR product. This material is then extruded automatically under approximately 300-1000 psi pressure using a hydraulic cylinder (Enerpac, Butler, WI) connected to a metering pump (Eldex, Menlo Park, CA). The RP/BR bead (approx. 2-mm dia.) is extruded into the custom-blown glass burn chamber where it is ignited to produce the RP/BR aerosol. A small envelope of nitrogen (N_2) gas is bled continuously into the RP/BR extrusion tip to prevent a backburn of RP/BR.

(3) Inhalation chamber subsystem.--The inhalation chamber is a standard stainless steel unit (91.5 x 91.5 x 91.5 cm) with autoclave door (Bertke and Young, Cincinnati, OH). The internal chamber has 3 shelves each containing 4 stainless steel wire mesh animal cages (30.5 x 30.5 x 30.5-cm). A PVC drain valve is plumbed to the bottom of the chamber for flushing the interior to a floor drain.

(4) Air-movement/-condition/-filtration subsystem.--As stated, air flow is caused by an in-line industrial vacuum (Dayton Electrical Mfg. Co., Chicago, IL). This vacuum source is located at the end of the closed, air-flow line. Calibrated air flow was determined using a Pneumotachograph Air Pressure Gauge (OEM Medical, Inc., Richmond, VA). The operator regulated a variable voltage auto transformer (Staco Energy Products, Dayton, OH) which controlled the vacuum source so that a stable 250 l/min flow of air was maintained through the System.

Conditioning of the intake air refers to humidification, filtration, and cooling. Humidification of the intake air was accomplished using a commercial console humidifier (Emerson Electric Co., St. Louis, MO) with a custom-fabricated Plexiglas humidity-collection chamber (61 x 30.5 x 30.5-cm) located over the humidifier's exhaust (Fig. 2). From the humidity-collection chamber, air was routed through flexible PVC tubing to a custom-made Plexiglas filter bay (33 x 33 x

15.3 cm). This bay collected the humidified air prior to passage through an Absolute Filter Unit (Young and Bertke Co., Cincinnati, OH) which contained a pleated, coarse filter (American Air Filter, Louisville, KY), a charcoal bed and a HEPA filter (Mine Safety Appliances Co., Pittsburgh, PA).

Next, the humidified, filtered air flowed through PVC tubing to the Hygrothermograph Chamber. This Chamber was custom-fabricated of Plexiglas (62.3 x 31.8 x 32.4 cm) and was designed to allow filtered air mixing along with RH measurement. A Hygrothermograph (Belfort Instrument Co., Baltimore, MD) was placed inside the chamber and the lid of the chamber was sealed. The Hygrothermograph was calibrated prior to each day's RP/BR burns using a fixed-position psychrometer (Belfort Instrument Co., Baltimore, MD). Humidity in the air-flow line was maintained at between 40 and 60 percent.

From the Hygrothermograph Chamber, air moved through flexible PVC tubing to the glass burn chamber. The aerosol-laden, heated air then moved from the burn chamber through flexible and rigid sections of stainless steel pipe to the apex of the inhalation chamber. A large portion of this pipe was surrounded by a water jacket; and, cold water was circulated between the jacket and a cold water bath (Massagerata-Werk Lauda, West Germany) to cool the intake pipe and the aerosol. Activation of this temperature-control subsystem was a decision of the operator. Generally, circulation of cold water was not required as long as the room temperature remained less than 21° C or if low-concentration RP/BR burns were planned (i.e., <3.0 mg/l).

After the aerosol-laden air reached the apex of the inhalation chamber, it dispersed throughout the chamber. A uniform flow was assumed to occur from apex to base. The aerosol was exhausted from the base of the chamber via PVC pipe. From the RH-recording port, the aerosol moved to a 7-bank, DX-grade coalescent filter unit (Balston Filter Products, Lexington, MA). The filter removed over 99 percent of the aerosol and associated contaminants from the chamber exhaust (Holmberg et al., 1985). Finally, the "scrubbed air" flowed to the vacuum source (Dayton Electrical Mfg. Co., Chicago, IL) via flexible PE tubing, and exited the System (building) via PE tubing through a ceiling vent. A 30-gallon PVC shroud covered the vacuum source to prevent any residual smoke products from entering the room.

b. Filtered-air Inhalation Chamber System

Figure 3 is a technical illustration of the Filtered-air Inhalation Chamber System. The insert of Figure 3 shows the general floor plan of the DWRC Laboratory (Building 16), with animal-housing areas and inhalation systems. The Filtered-air

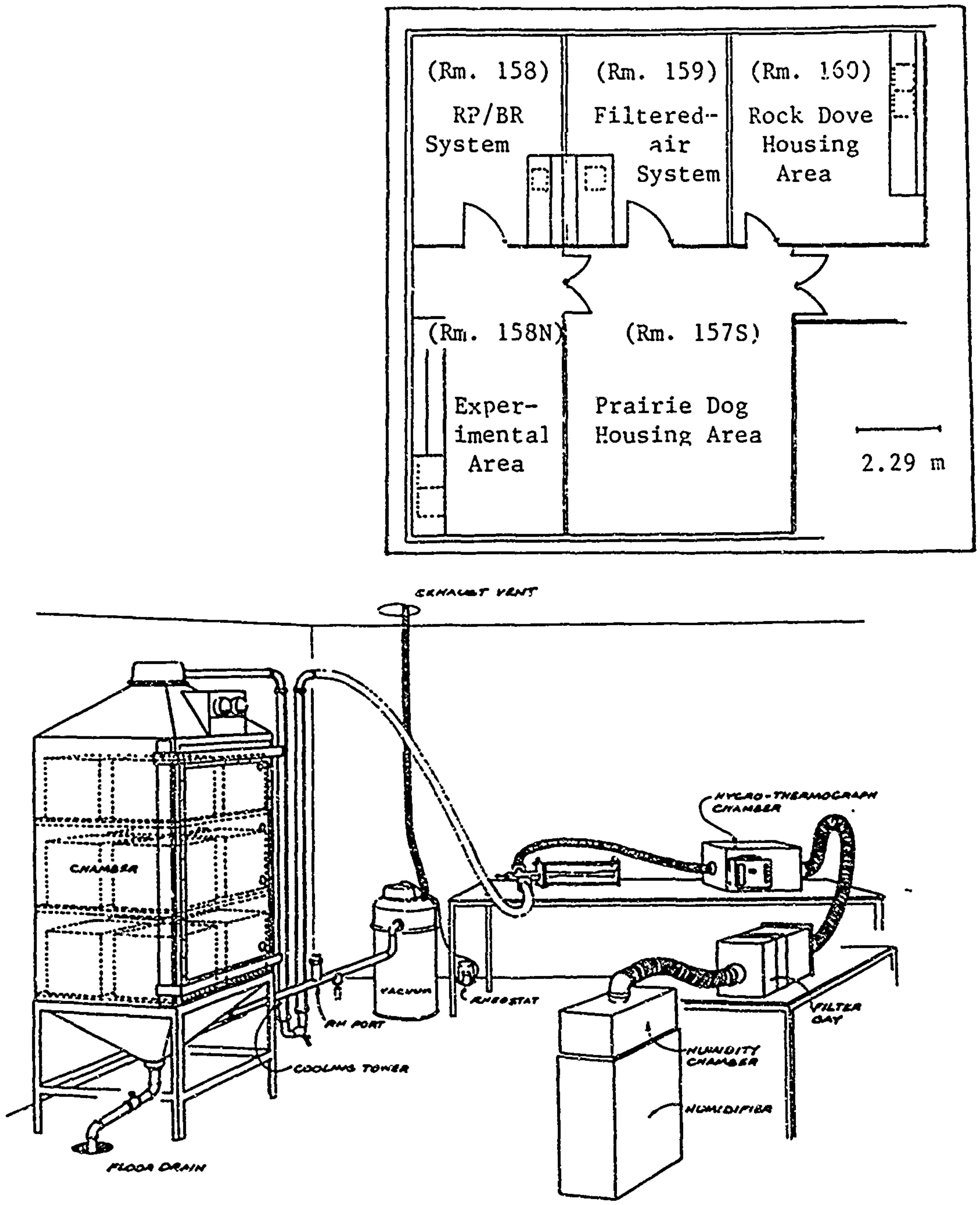

Figure 3. Technical illustration of the Filtered-air Inhalation Chamber System. (Note.--Components of the system are scaled relative to the perspective, i.e., 2.54 cm equals 0.6 m, but the locations of some components have been drawn to improve the visual display.) The insert shows a schematic drawing of the laboratory areas of DWRC housing the inhalation systems or the wildlife species during toxicity range-finding studies.

Inhalation Chamber System was used to expose separate groups of animals to equivalent durations of filtered air. This System was constructed to duplicate properties of the Modified RP/BR Extruder and Inhalation Chamber System, but without the generation of RP/BR aerosol. Identical equipment, tubing, and products comprised both Systems. Because the Filtered-air Inhalation Chamber System was not described in Sterner et al. (1988), details of this System are provided here.

Briefly, air flow was produced by an in-line vacuum (Dayton Electrical Mfg. Co., Chicago, IL). Air-flow rates were calibrated using a Pneumotachograph Air Pressure Gauge (OEM Medical, Inc., Richmond, VA); calibration procedures were identical to those used with the RP/BR System (see Sterner et al., 1988). Specific magnahelic readings equivalent to 250 l/min and 500 l/min flow rates were 0.35 and 1.20 in. of water, respectively.

Humidification of the intake air was accomplished using a commercial console humidifier (Emerson Electric Co., St. Louis, MO). A Plexiglas humidity-collection chamber (61 x 31.5 x 30.5 cm) was located over the humidifier's exhaust. The humidified air was routed through a 1.09-m length of 10.16-cm (i.d.) flexible PVC tube to a custom-made Plexiglas filter bay (33 x 33 x 15.3 cm) and an Absolute Filter Unit (Young and Bertke Co., Cincinnati, OH). This filter unit contained a pleated, coarse filter (American Air Filter, Louisville, KY), a charcoal bed and a HEPA filter (Mine Safety Appliances Co., Pittsburgh, PA).

Next, the filtered air flowed through a 1.22-m section of 10.16-cm (i.d.) flexible PVC pipe to the Hygrothermograph Chamber. This chamber was custom-made of Plexiglas (62.3 x 31.8 x 32.4 cm). The same Hygrothermograph (Belfort Instrument Co., Baltimore, MD) used for the RP/BR-aerosol exposures was placed into this chamber during measurement sessions. Humidity was added, as needed, to the intake air so as to maintain between 40 and 60 percent RH.

From the Hygrothermograph Chamber, air moved through a 1.57-m section of 7.5-cm diameter flexible PVC tubing to the glass burn chamber of a RP/BR extruder. That is, an extruder was placed in the air intake line, but was never loaded with RP/BR.

From the "blank" burn chamber, air flowed through a 2.47-m length of 5.6-cm diameter flexible stainless steel pipe. A U-shaped 5-m length of 6.35-cm diameter stainless steel pipe was run from the end of the flexible pipe to the apex of the inhalation chamber (i.e., intake pipe duplicated as in the RP/BR System). The base of the U-shaped column also was joined by a custom-molded stainless connector with a 5.6-cm (o.d.) valve and faucet (14.5-cm-long, 1.9-cm i.d.) to form the condensate drain on the RP/BR System. <u>No water jacket cooling column surrounded the stainless steel intake line of the Filtered-air System.</u>

Air then flowed down through the inhalation chamber, from apex to base. At the base of the chamber, the air was exhausted via a standard PVC pipe (5.08 cm o.d.). Similar to the RP/BR System, a special RH-recording port was located 24 cm from the chamber outlet (see Fig. 3). The RH port was made of a 20-cm length of 10.16-cm (o.d.) clear Plexiglas tubing plumbed vertical to the exhaust pipe. The port was sealed with a PVC end cap. At the end of each exposure session, a measurement of in-chamber RH was obtained by inserting a standard wet-/dry-bulb thermometer in the chamber exhaust line for approximately 5 min. Again, the RH was determined using standard charts corrected for barometric pressure (U.S. Department of Commerce, 1965).

From the RH-recording port, air moved to the vacuum source (Dayton Electrical Mfg. Co., Chicago, IL). No 7-bank, DX-grade filter unit was present in the Filtered-air Inhalation Chamber System. The air was then vented from the System and from the building through wire-ribbed PE tubing (5.02 cm dia.) via the room's ceiling vent. No 30-gal PVC shroud covered the vacuum source of this System.

3. RP/BR Aerosol and Filtered-air Monitoring

Single or multiple 80-min exposures of animals to selected target concentrations of RP/BR aerosol or equivalent durations of filtered air were the main experimental treatments used in the current studies. Characterization of the chamber atmosphere present for each RP/BR-aerosol or filtered-air exposure during the toxicity range-finding studies was accomplished using techniques described in the Task 1 Report (Sterner et al., 1987). Table 1 lists these variables and respective analytical methods. Figure 4 illustrates the chamber monitoring schemes associated with the Modified RP/BR Extruder and Inhalation Chamber System and the Filtered-air Inhalation Chamber System. It shows the complex sequence of atmospheric samples obtained to document the dosimetry associated with each burn.

Sampling of in-chamber conditions differed for the 2 chambers; no opacity readings were taken for the Filtered-air System. Checks of the Modified RP/BR Extruder and Inhalation Chamber System atmosphere involved 7 sets of variables: (a) aerosol mass, (b) phosphoric acid (H_3PO_4) titration, (c) aerosol opacity, (d) aerosol particle size, (e) respiratory gases, (f) contaminant gases and (g) temperature/humidity. Checks of the Filtered-air Inhalation System atmosphere involved 6 sets of variables: (a) aerosol mass, (b) phosphoric acid, (c) respiratory gases, (d) contaminant gases, (e) aerosol particle size, and (f) temperature/humidity.

a. Aerosol Mass

Aerosol mass collections were made using 45-mm-diameter acrylic filter holders (Phipps and Bird Co., Richmond, VA) and 45-mm-diameter Borosilicate-glass filter discs (Phipps and Bird

Table 1. List of variables, plus respective analytical techniques, used to characterize in-chamber conditions during the RP/BR or filtered-air exposures of the toxicity range-finding studies.

Variable	Technique
Aerosol Mass	Gravimetric Analysis
Phosphoric Acid (H_3PO_4)	Titration Analysis
Aerosol Opacity[a]	ORNL Infrared Detector
Aerosol Particle Size	QCM Cascade Impactor
Respiratory Gases	
Oxygen (O_2)	Gastec Analyzer Tube
Carbon Dioxide (CO_2)	Gastec Analyzer Tube
Contaminant Gases	
Carbon Monoxide (CO)	Gastec Analyzer Tube
Phosphine (PH_3)	Gastec Analyzer Tube
Hexane (C_6H_{14})	Gastec Analyzer Tube
Temperature/Humidity	
Temperature	Digital Thermometer
Relative Humidity	Wet-/Dry-bulb Thermometer

[a] Although digital counts of aerosol density were obtained for each RP/BR burn, these data were not summarized for analysis. Aerosol opacity charts were used to estimate maximum steady-state concentrations achieved over 36 to 40 min samplings on a portion of the exposure sessions as outlined in Appendix G.

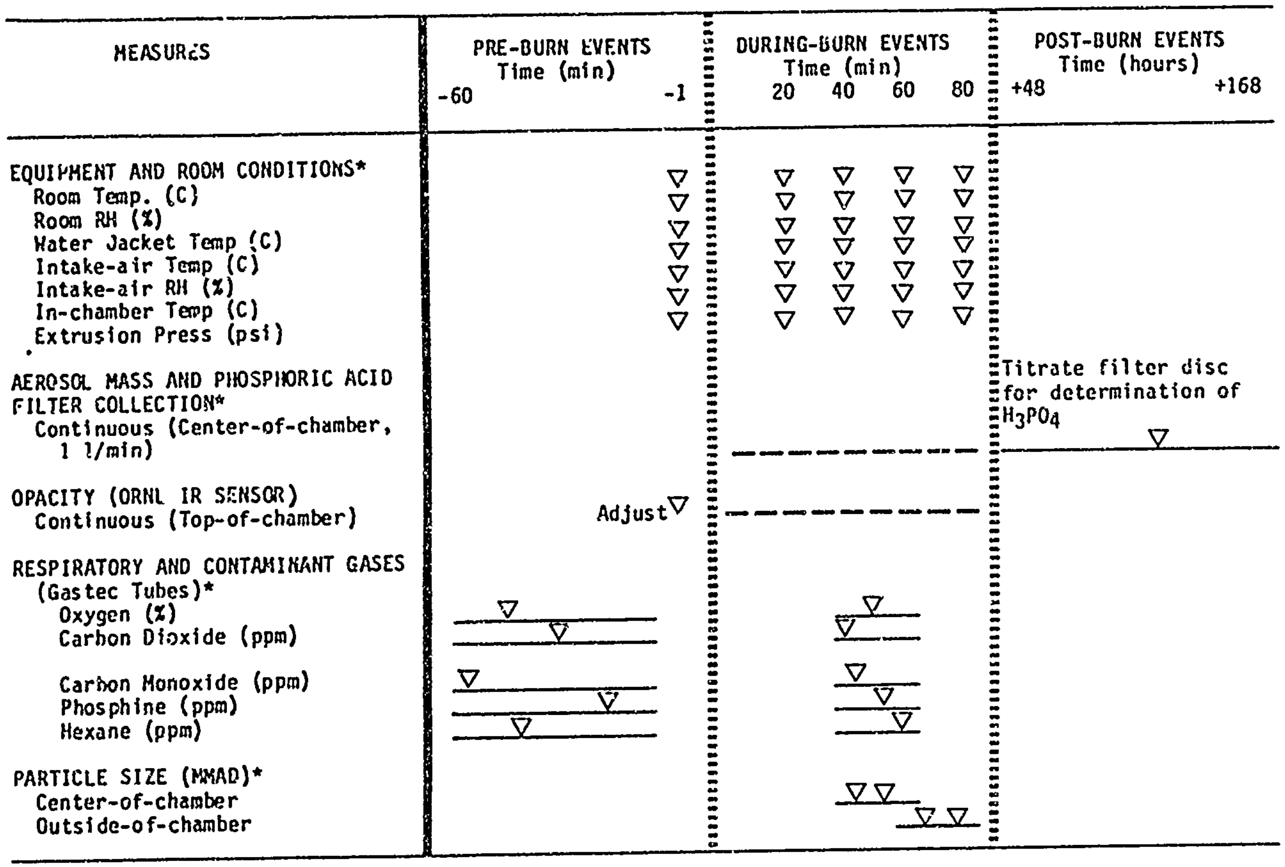

Figure 4. Schematic illustration showing the extensive sampling schedule used to monitor RP/BR and filtered-air atmospheres within chambers for each exposure of the toxicity range-finding studies. (Note.--"Arrows" designate discrete-type measurements collected at fixed times; "arrows above solid lines" designate discrete-type measurements collected at fixed, but variable, times within the period represented by the line; and "dashed lines" represent continuous sampling of aerosol or opacity.) The measures marked with an asterisk (*) were collected for the Filtered-air Inhalation Chamber System according to the same schedule indicated here for RP/BR exposures; opacity monitoring was not conducted during the filtered-air (control) exposures.

Co., Richmond, VA). A 45-mm-diameter Buna-n Rubber O-Ring was used to seal the filter discs within the filter holders. The filter holders were machined and threaded to a 0.625 in. diameter at the center to hold a Millipore Limiting Flow Orifice (Millipore Corp., Bedford, MA); these orifices provided a uniform flow rate of 1 l/min $\pm$ 5% for sampled aerosol. During collection, a filter holder was mounted onto the downstream leg of interconnected plastic and PE tubing running from the center of each chamber to a vacuum pump. In-line sampling connections were made air-tight with Teflon tape.

Collection of aerosol mass samples for the RP/BR exposures involved the use of a special Aerosol Sampling System (Fig. 5). This System was described in Sterner et al. (1988). Briefly, sections of rigid plastic tubing and pliable PE tubing were connected between the center of the chamber and a filter holder via a sampling port on the right front of the inhalation chamber. The filter holder was then connected to a stainless steel tri-valve (Whitey Co., Highland Heights, OH) on a custom-built panel (Fig. 5). The tri-valve was used to direct vacuum pressure across a fiberglass filter disc during RP/BR aerosol data collections. Vacuum pressure for the aerosol sampling system was created by a Millipore Vacuum Pressure Pump (Millipore Corp., Bedford, MA) attached to the tri-valve.

Filtered-air mass collections were made using essentially the same type of equipment, except that the Aerosol Sampling System was not used. A direct-line connection of plastic and PE tubing connected the center of the filtered-air chamber with a filter holder and the Millipore Vacuum Pressure Pump (i.e., 1 l/min sampling rate).

Aerosol mass of both RP/BR-aerosol and filtered air was measured gravimetrically. This involved weighing the assembled filter holder, filter disc, and O-ring on a Sartorius analytical balance (Brinkman Instruments Co., Westbury, NY) immediately prior to, and then following each RP/BR aerosol or filtered-air collection. The difference between the pre- and post-weight of filters was the mass of aerosol or filtered air accumulated during the sampling period (i.e., 80-min exposure).

b. Phosphoric Acid (H_3PO_4) Titration

Titration analysis was used to determine the amount of H_3PO_4 contained on each filter-disc. This measure is considered representative of the total phosphorus content of the aerosol (Burton et al., 1982).

Following gravimetric analysis, filter discs collected for respective sampling periods were deposited into covered plastic Petri dishes (Miles Laboratories Inc., Naperville, IL). Single discs were collected at RP/BR aerosol target concentrations below

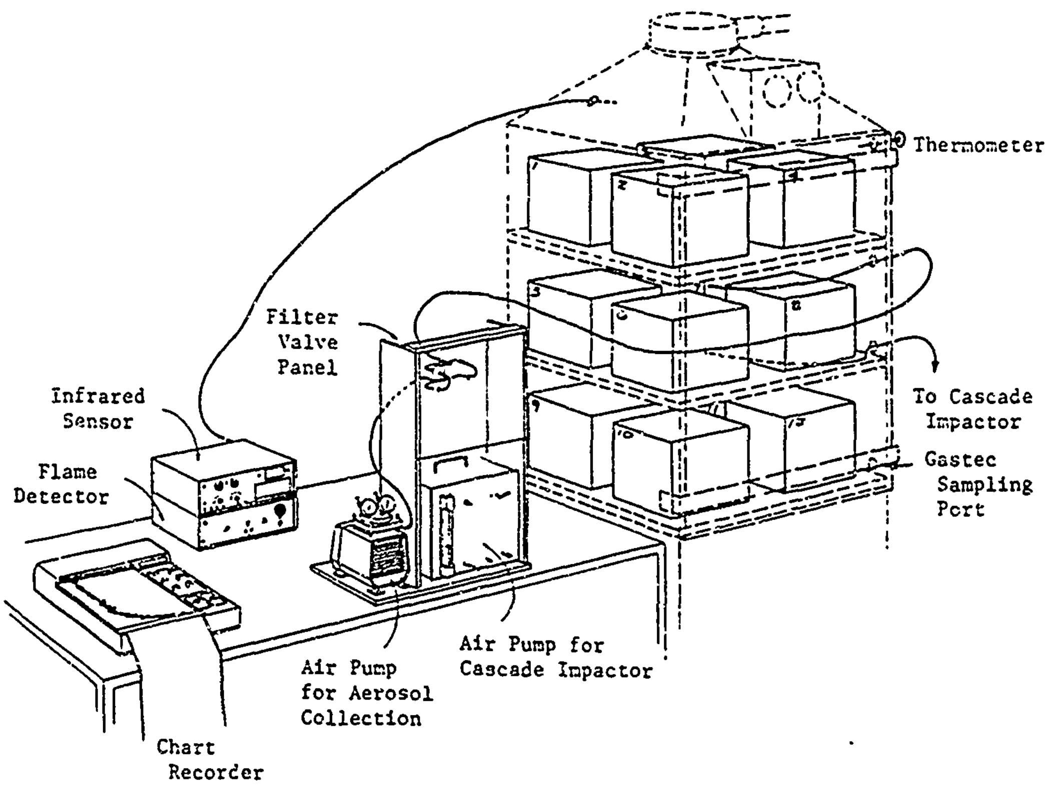

Figure 5. Technical illustration of the Aerosol Sampling System used to collect RP/BR aerosol mass - H_3PO_4 titration filters, opacity measurements and particle size measurements during toxicity range-finding studies. (Note.--Cage 12 was unused throughout Task 2; room air drawn into the chamber through the Gastec Sampling Port was believed to cause a localized dilution of RP/BR aerosol for Cage 12.)

4.0 mg/l; whereas, two successive discs were collected at target concentrations of 4.0 and higher to avoid saturation of the discs. The Petri dishes were stored in a ventilated cabinet for between 48 and 168 h to allow for complete hydrolysis of the acids. This aging process was used because, at the time of aerosol collection, there were several acids present (i.e., H_3PO_4, polyphosphorics and cyclic polyphosphorics). With storage, the larger phosphoric acid molecules hydrolyze to H_3PO_4 (Burton et al., 1982). A number of unused, "blank" discs, exposed to ambient room air during RP/BR-aerosol and filtered-air sessions, were also stored in this manner for purposes of quality assurance analyses.

Titration analysis involved the use of a Radiometer DTS-800 Multititration System (Radiometer America Inc., Cleveland, OH). Upon removal of the filter from storage, each disc was extracted using 60 ml of boiled deionized water in a 400-ml glass beaker and agitated with a magnetic stir bar for 10 min. When two pads were involved, the solutions were combined after extraction. Subsequently, a 20-ml sample of the extracted solution was pipeted into a 22- to 45-ml disposable sample cup (Radiometer America Inc., Cleveland, OH) and was titrated using either a 0.1N or a 0.01N sodium hydroxide (NaOH) titrant (Fischer Scientific, Fair Lawn, NJ). The titrator was programmed to calculate mg of H_3PO_4 in the total extracted sample by inflection-point titration. The formula used to make the calculation for single filter pads was:

$$\text{Total mg } H_3PO_4 = \frac{(\text{ml titrant to 1st inflection})(\text{meq/ml titrant})}{\text{ml of sample}} \times \text{Factor}$$

where <u>Factor</u> refers to a unique titration constant based on (3 x ml of sample x formula wt in mg/meq of H_3PO_4). Assuming that hydrolization to H_3PO_4 is complete, the first inflection point is a direct measure of the total number of phosphorus atoms (i.e., mg of H_3PO_4) present in the extracted sample. If only H_3PO_4 is present, the amount of NAOH required to titrate to the first inflection point is equal to the NAOH needed to titrate from the first to the second inflection point. Comparisons of H_3PO_4 standard solutions indicated that all aerosol filters had sufficiently hydrolized.

c. Aerosol Opacity

The density of RP/BR aerosol within the inhalation chamber was monitored continuously during the burns using an ORNL Aerosol Sensor (Higgins, Gayle and Stokely, 1978; Holmberg et al., 1985). This sensor consisted of an infrared light-emitting diode mounted beside, but optically separated from, a phototransistor. Aerosol particles scatter the infrared light, raising the mv output of the transistor and providing an analog record on a chart recorder (Cole Palmer Instrument Co., Chicago, IL). The sensor consisted of a 16-cm-long metal probe which was inserted into the top of the inhalation chamber so as to minimize interference due to animal cage reflectance back to the sensor probe (see Fig. 5).

Each sensor probe had its own characteristic sensitivity. Each probe was also calibrated by comparing gravimetric filter samples of the aerosol taken over a known time period with the integrated sensor response provided by a digital counter on the readout module.

Charts of the opacity sensor measurements and the total integrated sensor counts for each RP/BR burn have been archived. These charts provide visual records of flame outs and stability of combustion product aerosol levels during each burn and the charts were also used to graphically estimate steady state concentrations as outlined in Appendix G.

d. Aerosol Particle Sizes

Samples for determining aerosol particle size were obtained between 20 to 60 min after ignition of the RP/BR material. Measurements were derived by using a Piezo-electric Quartz-Crystal-Micro-Balance (QCM) Cascade Impactor (California Measurements Inc., Sierra Madre, CA).

The procedure involved 2 successive RP/BR- aerosol samplings from near the center of the chamber, along with 2 samples of the room air taken immediately after the RP/BR-aerosol or the filtered-air readings. Aerosol sampling involved connecting a plastic sampling tube to the ORNL vacuum flow pump and to the high concentration slide valve on the QCM Cascade Impactor. Interconnected lengths of PE and rigid plastic tubing then connected the slide valve of the Cascade Impactor to the center of the inhalation chamber. The aerosol sampling flow rate was 4.3 l/min, and an in-line 45-mm-diameter aerosol filter protected the pump's diaphragm from phosphoric acids. During room air measurements, samples of ambient air were collected from outside of the inhalation chamber (Rooms 158 or 159).

A 10-sec sample of aerosol or air for each respective measurement was circulated a minimum of 90 sec within the impactor column. Injections of aerosol or air were drawn into the stack of matched frequency quartz crystal oscillator pairs using the slide valve. Upon completion of this temporal sequence, paper tape output containing (1) a histogram of the relative mass of aerosol detected for each stage, (2) the number of 300 μl samples taken, (3) the total aerosol mass (mg/m^3) accumulated on all stages, and (4) the change in frequency (Hz) and mass accumulated (mg/m^3) for each stage, was printed. Actual determinations of MMAD and geometric standard deviation for each sample were completed using a graphical procedure whereby the cumulative normalized percentage of total mass detected was plotted for the particle size range limit of each impactor stage as outlined by Chuan (1986).

e. Respiratory Gases

Oxygen and CO_2 levels within the inhalation chamber were measured during each exposure throughout Task 2. This involved use of the Gastec Gas Detection System (Gastec Inc., Newark, CA)--a standard industrial-hygiene-type analyzer tube and pump system (see Appendix B). Oxygen and CO_2 were measured using Gastec Analyzer Tubes +31 (% O_2) and 2LL (ppm CO_2), respectively. Sampling was conducted immediately preceding, and between 20 and 60 min following, ignition of the RP/BR or start of the filtered-air exposure. A port located on the bottom-right side of each inhalation chamber allowed for insertion of the analyzer tube and the sampling of either aerosol or filtered air. Upon completion of the sampling sequence, the tube was withdrawn, the sampling port was sealed with the rubber cone-shaped plug, and the farthest migration of dye was determined using the graduated markings on the side of the analyzer tube. Actual percent O_2 and ppm CO_2 were corrected for atmospheric pressure at 1646 m (5400 ft) elevation based upon the following formula:

$$\text{Corrected Analyzer Tube Value} = \text{Actual Tube Value} \times \frac{760 \text{ mm Hg}}{628 \text{ mm Hg}} .$$

f. Contaminant Gases

Determination of the amounts of CO, PH_3 and C_6H_{14} were performed using identical procedures as those described for respiratory gases. Gastec Analyzer Tubes 1LL (CO), 7L (PH_3) and 102L (C_6H_{14}) were used (see Appendix B). Samples were drawn immediately preceding, and within 20 to 60 min after, ignition of RP/BR or start of the filtered-air exposure. The pre-samples were collected to verify that no residual contaminants were present prior to exposure. Data were again corrected for atmospheric pressure at 1646 m (5400 ft) elevation using the formula:

$$\text{Corrected Analyzer Tube Value} = \text{Actual Tube Value} \times \frac{760 \text{ mmHg}}{628 \text{ mmHg}} .$$

g. Temperature/Humidity

In-chamber temperatures were monitored at successive 20-min intervals throughout each exposure trial using a VWR Digital Thermometer (Van Waters and Rogers, Denver, CO). The thermometer was permanently mounted into a port at the top right-front side of each chamber.

A special RH-recording port in the main air exhaust line exiting the inhalation chambers permitted assessment of in-chamber RH (see Figs. 2 and 3). A standard wet/dry bulb thermometer was inserted into the port for approximately 5 min before the end of each RP/BR burn or filtered-air exposure, and the RH was then determined using standard charts corrected for barometric pressure (U.S. Department of Commerce, 1965).

4. RP/BR-aerosol and Filtered-air Exposure Conditions

Precise estimates of doses of RP/BR aerosol inhaled by the animals are, of course, impossible. Numerous unknown factors such as respiration rates, ventilatory exchange volumes or ingestion via grooming can affect dose delivery levels in whole-body exposure studies. The accepted practice is to describe physical and chemical properties of the atmospheric conditions present within the inhalation chambers during each RP/BR-aerosol and filtered-air exposure.

a. RP/BR-aerosol Dosimetry

Figure 6 presents a tracing of the within-chamber opacity chart obtained using the ORNL infrared sensor for a burn at an RP/BR extrusion pump setting of 180 μm with 250 l/min air flow. Note that opacity follows a 3-phase pattern during the approximately 80-min exposure trial. The phases can best be described as: (1) an approximately 10-20 min period of increasing RP/BR-aerosol concentration during which the chamber is filled with aerosol, (2) an approximately 40-50 min period of relatively asymptotic, maximal aerosol concentration, and (3) an approximately 20-min period of decreasing aerosol concentration during which the RP/BR product is extinguished and the chamber is vented of aerosol. The total dose of aerosol inhaled by each animal was affected by this 3-phase RP/BR-aerosol concentration pattern. In Task 2, aerosol collections for both gravimetric and H_3PO_4 titration analysis were obtained throughout this 80-min session; this differed from Task 1 (Sterner et al., 1988), where filter samples were collected for only 60 min. An estimate of the maximal, steady-state concentration of each exposure was calculated using a graphic analysis as indicated in Appendix G.

b. Available RP/BR-aerosol and Air Quality Data

Results of Task 1 aerosol uniformity tests showed that the Modified RP/BR Extruder and Inhalation Chamber System provided for the reliable exposure of animals to uniform concentrations (i.e., ≤20% maximal heterogeneity) of RP/BR aerosol, with satisfactory air quality present throughout all 1-h exposures (Sterner et al., 1988). Two limitations to aerosol acceptability were: (1) Cage Site 12 (i.e., bottom, right-front side of chamber; see Fig. 4) yielded a reduced RP/BR-aerosol concentration, and was to be excluded from use during Tasks 2 and 3 and (2) high levels of CO (i.e., ≥35 ppm) were observed during burns conducted at the 270 μm extrusion pump setting with a 250 l/min air flow rate. Although this result was later used to set the maximum extrusion rate at a pump setting of 180 μm in Task 3, settings as high as 293 μm were used during range-finding studies

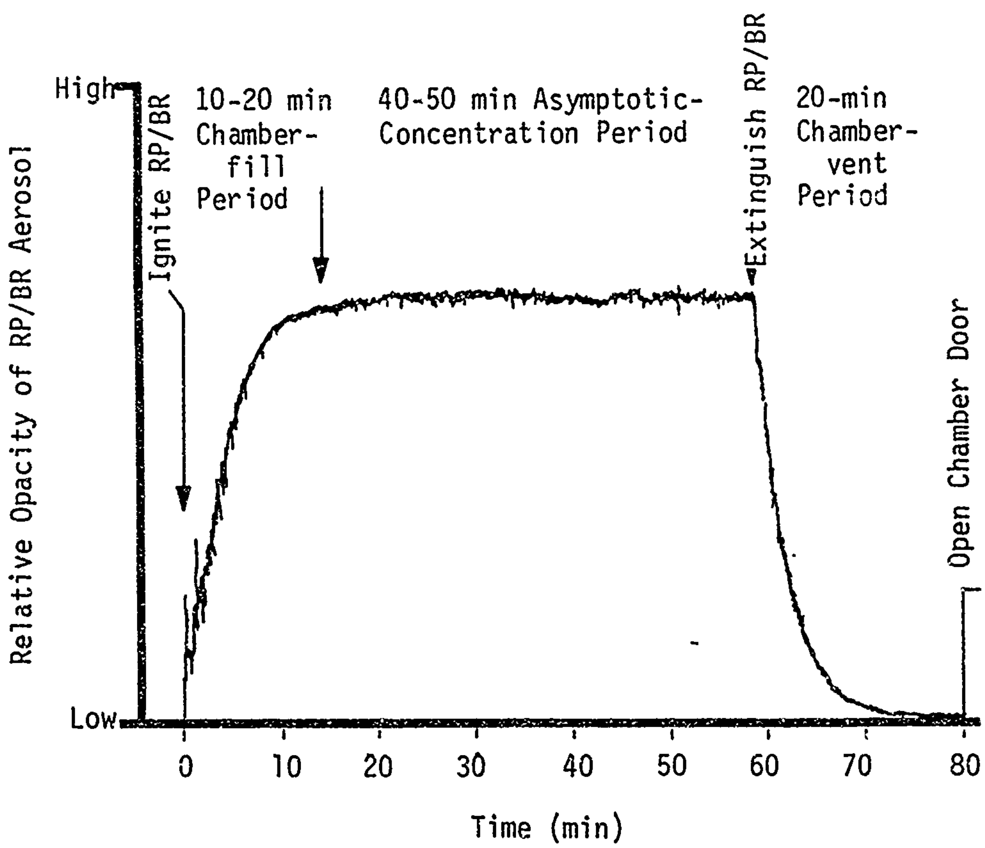

Figure 6. Tracing of the ORNL infrared sensor recording for a representative RP/BR burn at the 180 μm extrusion pump setting and a 250 l/min air flow rate. (Note.--This burn yielded a total aerosol mass value of 252.7 mg or 3.16 mg/l average RP/BR concentration for the 80-min exposure; the plot of relative opacity demonstrates the chamber concentration changes associated with the 80-min exposure session.)

because these high CO levels were unconfirmed at the start of Task 2.

Additionally, on 17-18 August 1987, staff of the Bio/Organic Analysis Section, Analytical Chemistry Division, ORNL, performed an independent characterization study of the atmosphere produced in the Modified RP/BR System (Moneyhun, Moody and Jenkins, 1988; see Appendix C). This study was performed as part of our quality assurance monitoring program, with the work authorized under APHIS IAG 87-74-01. Results of this study were important because of the timing of the work -- the ORNL checks were conducted approximately midway through the current toxicity range-finding studies (i.e., 29 June to 11 November 1987). Results of the ORNL study confirmed the satisfactory nature of RP/BR aerosol produced in the Modified RP/BR System, with practically all aerosol and air quality data (i.e., except C_6H_{14} levels) confirming those reported by Sterner et al. (1988) at equivalent extrusion and air flow rates.

C. Toxicity Effects in Black-tailed Prairie Dogs

1. Procedures

a. Sub-study Treatment Assignment

A total of 72 out of 110 captured prairie dogs was used in the toxicity range-finding study. The research further involved 2 sub-studies consisting of an initial range-finding study with 48 prairie dogs and an additional sub-study with 24 prairie dogs.

In the initial sub-study, 48 animals (24 of each sex) were randomly selected from the 110 captured prairie dogs. Animals of each sex were then rank-ordered by body weight. Three weight classes were arbitrarily developed for each sex by assigning the 8 lowest weight prairie dogs to the "light" group, the next 8 to the "medium" group, and the heaviest 8 to the "heavy" group. Light weight males ranged from 733 to 911 g, medium weight males ranged from 916 to 1019 g, and heavy weight males ranged from 1029 to 1189 g. For females, these respective weight ranges were 634-795 g, 803-891 g, and 917-1099 g. Next, 1 male and 1 female prairie dog from each of the light, medium and heavy weight groups (i.e., 3 males and 3 females) were randomly assigned to 1 of 8 treatment groups.

Due to the lack of observed RP/BR-smoke effects in this initial sub-study, 4 additional groups were exposed for more sessions and were examined 4 months later. In this second sub-study, 24 prairie dogs (12 of each sex) were randomly selected from the 62 remaining animals. The same rank ordered weight procedure as cited earlier was used to assign animals of each sex to light, medium and heavy weight categories. The weight ranges of these males were generally increased as compared to the initial sub-study (i.e., direct comparisons on this factor were confounded). Weight ranges of the males in the respective weight categories were: 947-1116 g, 1170-1244 g and 1279-1516 g; weight ranges for light, medium and heavy weight females were: 875-933 g, 1012-1054 g and 1071-1276 g, respectively. Again, 1 male and 1 female prairie dog were randomly selected from each weight class (i.e., 3 males and 3 females) and were assigned to 1 of 4 treatment groups. Treatments of RP/BR aerosol exposure involved use of the Modified RP/BR Extruder and Inhalation System (Fig. 2), and control treatments of filtered air involved use of the Filtered-air Inhalation System (Fig. 3).

Six of the 8 initial sub-study groups were to receive 1 or 2 successive daily exposures to RP/BR-aerosol target concentrations of 2.0, 4.0 and 6.0 mg/l (i.e., extruder pump settings of 78, 180 and 293 µm, respectively); whereas, the remaining 2 groups were to receive 1 or 2 successive daily exposures to filtered air of durations equal to the longest daily RP/BR-aerosol exposure of the previous groups.

Two of the 4 second sub-study groups were designated to receive either 3 or 4 successive daily exposures to RP/BR-aerosol target concentrations of 6.0 mg/l (i.e., extruder pump setting of 293 µm), and 2 groups were designated to receive 4 successive daily exposures to filtered air of durations equal to the longest daily exposures of the above RP/BR-smoke-exposed groups. One of the filtered-air groups was given "regular handling" with respiration checks at rest and post exercise, and the other group was given "minimal handling" in checks for respiratory congestion. This second filtered-air group served as a control for any possible organ pathology induced by repeated animal restraint.

b. Assessment Paradigm

Within each sub-study, toxicity assessments adhered to a standard paradigm. That is, each sub-study was from 38 to 41 days in length, with differences in length due to certain groups receiving between 1 and 4 exposure days. The paradigm consisted of a 7-day Pre-exposure Phase (Baseline), a 1- to 4-day Exposure Phase, and a 28-day Post-exposure Phase. Three sets of toxicity-assessment variables were measured at specific times within these Phases: Symptomatology/Mortality, Gross Necropsy and Histology. Specifically, mortality was assessed daily throughout each Phase. Symptomatology was measured daily during the Pre-exposure and Exposure Phase, and on Days 1-7 (Post 1) along with Days 10, 13, 16, 19, 22, 25 and 28 (Post 2) of the Post-exposure Phase. All animals were euthanized via i.p. injection of sodium pentobarbitol 31 days after their last exposure, with the gross necropsy examinations of 10 organs conducted immediately after death, and tissue sections of 7 of the organs were taken for later histological examination by APHIS pathologists (NVSL, Ames, IA).

c. Symptomatology/Mortality

Table 2 presents a list of the 8 symptomatology/mortality categories, plus respective operational definitions or procedures used to evaluate RP/BR-smoke effects during the 2 toxicity range-finding sub-studies. Note that the list is classified according to 5 qualitative measures (i.e., body posture, respiratory congestion, coat condition, aggression and vocalization) and 3 quantitative measures (i.e., water consumption, body weight and mortality).

As mentioned, mortality was determined daily throughout the course of each sub-study during an initial check of each animal's cage by an investigator. This was conducted between 0800 and 0900 h MST, immediately prior to the symptomatology examinations.

Symptomatology assessments were performed on animals in each group throughout the Pre-exposure, Exposure and Post-exposure Phases. The typical symptom examination required 3 to 5 min per animal. Each examination involved the following sequence of events: (a) a visual determination of body posture and coat condition while the prairie dog was in the home cage, (b) removal of the animal from the home cage and determination of respiratory congestion (rest) and aggression, (c) placement of the animal into a large, ventilated, pre-tarred metal container and measurement of body weight, (d) placement of the prairie dog into a motorized stainless steel activity wheel (30 cm/sec rotation speed) for 60 sec, with the animal prodded to run by hand, (e) removal of the prairie dog from the wheel with an immediate determination of post-exercise respiratory congestion and aggression, and (f) placement of the prairie dog back into the home cage, with a subsequent determination of post-exercise body posture. Water consumption was measured for all animals simultaneously at the end of the symptom examinations for all groups.

To evaluate consistency of symptomatology scores, 18 prairie dogs were independently rated on the same day during the Pre-exposure Phase by 3 different investigators. Inter-observer reliability was later assessed by calculating the percentage of agreement among the ratings for each symptom category.

d. Gross Necropsy

On Day 31 post exposure, all 48 animals were euthanized with Beuthanasia-D Special solution (sodium pentobarbitol; Schering Corp., Kenilworth, NJ) injected i.p. Post-mortem necropsy examinations included assessments of the following organs by APHIS Veterinary Services personnel: nasal passages, trachea, larynx, epiglottis, bronchi, lungs, heart, liver, spleen and kidneys (see Appendix D - Standard Gross Necropsy Form).

Table 2. List of the 8 symptomatology/mortality categories, plus the operational procedures/definitions, used to rate each prairie dog during the 7-day Pre-exposure, 1- to 4-day Exposure and 14-session Post-exposure Phase (i.e., Days 1-7, 10, 13, 16, 19, 22, 25 and 28).

Symptom Category	Operational Procedure/Definition
Qualitative	
Body Posture (rest & post-exercise)	The body posture of each prairie dog was rated while at rest in the home cage and after 60 sec of mild exercise in a motorized stainless steel activity wheel (100 cm dia x 20.6 cm wide). The wheel rotated at approximately 30 cm/sec during exercise. Abnormal ratings were assigned to animals that remained in a prostrate position with their abdomen against the cage floor or remained "hunched over" with head down. Positive ratings were assigned to prairie dogs that displayed these postures even though prodded gently on the back (3-5 cm above the base of the tail) with a pen.
Respiratory Congestion (rest & post-exercise)	Respiratory congestion was checked before and after exercise using a stethoscope (Propper Mfg. Co., Long Island City, NY). Abnormal ratings were assigned to prairie dogs that displayed harsh rasping, gurgling, whistling, wheezing or buzzing sounds from the chest that were associated with movements of the chest or nostrils.
Coat Condition	The coat condition of each prairie dog was rated prior to exercise as groomed or ungroomed. Positive ratings for ungroomed were assigned to coats that had matted, wet-looking, gnarled or raised-up hair patterns. The symptom was distinguished from shedding, uneven and thinning hair.
Aggression	The aggression that each prairie dog displayed toward handlers was scored as high, medium or low. Positive ratings were assigned to prairie dogs that persisted in biting and growling during the symptom examination.
Vocalization (Occurrence and Quality)	The occurrence (present, absent) and quality of vocalizations (normal, affected or lost) were noted for those animals that barked during the examination session. An occurrence of vocalization was recorded if an individual prairie dog was observed emitting sounds while being examined during a daily session. For quality ratings, normal vocalization was a series of 8-20 short chattering-type barks or shrill yips. Affected vocalization was a low pitch and low volume bark/yip, with a raspy (laryngitis-type) sound. Lost vocalization was characterized by a "rushing air sound" during attempted barks/yips (i.e., little or no sound was emitted, similar to severe laryngitis).
Quantitative	
Water Consumption (24 h)	The daily water consumption of each prairie dog was measured by subtracting the pre- and post-weight (nearest g) of each water bottle after approximately 24 h (i.e., time of water availability recorded to nearest min). Bottles were removed, weighed, rinsed, refilled, reweighed and reattached to respective cages at the end of each symptom measurement session. Differences in the time of water availability were adjusted for a 24-h base using the formula: $$\text{24 h Consumption} = \frac{\text{24 (Measured water drunk in g)}}{\text{(Duration of availability in h)}} .$$
Body Weight	The daily or session (Post exposure) body weight of each prairie dog was recorded (nearest g) using an electronic balance (Model PE 3600; Mittler Corp., Highstown, NJ).
Mortality	Death (i.e., lack of respiration and heartbeat) was determined daily for each prairie dog during an initial check of the cages by an investigator (i.e., 0800-0900 MST).

e. Histological Examinations

Sections of the nasal passages, bronchi, lungs and liver, plus the entire trachea, larynx and epiglottis from each animal were preserved jointly in a specimen jar containing 10 percent formalin solution. Jars were numbered consecutively, and the respective jar number and animal number was logged onto a record sheet. The sample jars were then shipped to the NVSL, APHIS-VS, Ames, IA, for histological evaluation. Tissue specimens were prepared and examined for pathological signs according to standardized procedures used by staff of the Pathobiology Laboratory, Parasitology and Clinical Pathology Section, NVSL (see Appendix E).

The identities of the "filtered-air control specimens" were provided to NVSL Pathologists; these samples were then examined and they formed the basis for judging cellular and tissue abnormalities. After all tissue specimens had been examined, the group assignment of each animal was also given to the NVSL pathologists. This information was provided to enhance their detection of potential pathology and to aid in their reporting of clinical results.

f. Designs and Data Analyses

Symptomatology, mortality, gross necropsy and histopathology data were evaluated using both descriptive and inferential statistical methods. Regarding symptomatology, pre-exposure data served as a basis for comparison of frequency counts of treatment-induced symptoms. For the other measures, data from the filtered-air groups served as the basis of comparison for effects in RP/BR-aerosol groups. Tabular and graphical descriptions of the frequencies of positive symptoms observed for each group were prepared to illustrate specific changes in these measures (e.g., congested respiration, lost vocalization) among groups and phases.

Daily water consumption and body weight data were each analyzed for post-exposure effects using a 4-factor design involving a 4 (Concentrations) x 2 (Exposures) x 2 (Sexes) x 15 (Sessions) analysis of variance (ANOVA), with Sessions treated as a repeated measures factor (Winer, 1971). Data for the last daily session of the Pre-exposure Phase were compared with data for 14 sessions of the Post-exposure Phase.

A second pair of ANOVAs was used to determine whether a greater number of repeated daily exposures (1, 2 or 4) per se would lead to greater reductions of body weight and changes in water consumption in the RP/BR-aerosol groups as compared to the filtered-air (control) groups. This was also a 4-factor repeated measures design that involved the following: 2 (0.0 and 6.0 mg/l Concentrations) x 3 (1, 2 or 4 Exposures) x 2 (Sexes) x 15

(Sessions), with Sessions treated as a repeated measures factor (Winer, 1971).

All ANOVAs were computed using the PROC GLM Program of the SAS package of programs (SAS Institute, Inc., 1985) and Type III sums of squares to determine effects. Effects were tested at the 0.05 level of significance. Where significant effects were found, post hoc Duncan Multiple Range Tests were used for pair-wise comparison of all means (Waller and Duncan, 1969).

The gross necropsy and histological data for prairie dogs were treated descriptively. That is, percentages of specimens that showed various types of clinical pathologies were computed for RP/BR-exposure groups and for the filtered-air groups (see Appendix E).

2. Results and Discussion

a. Aerosol and Filtered-air Measurements

Table 3 presents medians and ranges of the aerosol and air quality variables used to characterize chamber conditions in range-finding studies with prairie dogs. The first 8 columns contain statistics for the 8 RP/BR groups; these groups were exposed to aerosol produced at the 78, 180 and 293 m extrusion pump settings (i.e., 2.0, 4.0 and 6.0 mg/l target concentrations, respectively) over 1 to 4 successive daily sessions. The last 4 columns present statistics for the 4 filtered-air (control) groups; these groups received 1, 2 or 4 successive filtered-air exposures equal in duration to the longest RP/BR aerosol exposure for each respective day.

Table 3 reveals that total aerosol mass and H_3PO_4 for the 8 RP/BR-aerosol groups was of acceptable uniformity. Median aerosol mass values varies $\leq$19 percent in multiple exposure sessions across burns conducted at respective extrusion pump settings. Calculated steady-state aerosol concentrations were found to be very close to the target concentration values. The H_3PO_4 titration data are essentially similar to those for aerosol mass. As expected, H_3PO_4 depositions are consistently two-thirds to three-fourths those of the total aerosol mass--results in agreement with prior research (Sterner et al., 1988; Moneyhun et al., 1988).

The particle size data also represent an important aspect of RP/BR-aerosol quality. All MMAD measurements show excellent agreement with those observed in other studies (Sterner et al., 1988; Moneyhun et al., 1988), and all values are <1.0 μm--values easily within the respirable range (Phalen, 1984).

Table 3. Median and range statistics of the aerosol and air quality variables characterizing the single or multiple RP/BR and filtered-air (control) exposures conducted at the 78, 180 and 293 μm extrusion settings for the toxicity range-finding studies with prairie dogs in Task 2.[a]

Target Concentration	2.0 mg/l		4.0 mg/l		6.0 mg/l				0.0 mg/l			
Extrusion setting	78 μm		180 μm		293 μm							
Exposures / Variable	1	2	1	2	1	2	3	4	1	2	4f	4f
Aerosol												
Aerosol Mass (mg)	93.3	112.3 (110.4-114.9)	262.1	264.9 (256.0-273.8)	321.1	394.9 (366.6-423.2)	376.0 (368.5-593.3)	366.2 (345.9-399.2)	3	1.7 (1.5-1.9)	11.5 (-1.6-25.0)	16.5 (5.0-33.0)
Aerosol Mass Concentration (mg/l) 80-min	1.2	1.4 (1.4-1.4)	3.4	3.1 (3.0-3.2)	4.2	4.6 (4.2-5.0)	4.4 (4.4-7.0)	4.6 (4.2-5.4)	--	.06 (.02-.2)	.14 (0.-.28)	.22 (.06-.45)
Steady-state Concentration (mg/l)	1.3	1.8 (1.8-1.8)	4.3	4.4 (4.3-4.4)	5.9	6.5 (5.9-7.1)	6.3 (5.9-9.4)	5.9 (5.6-6.8)	--	--	--	--
H_3PO_4 Titration (mg)	71.2	82.0 (78.0-85.9)	198.2	189.0 (182.8-195.8)	241.1	256.3 (249.0-263.6)	269.9 (258.2-283.9)	253.7 (234.8-269.1)	ND	ND	ND	ND
H_3PO_4 Concentration (mg/l)	1.0	1.02 (.98-1.05)	2.6	2.22 (2.15-2.29)	3.1	2.98 (2.96-3.0)	3.3 (3.0-3.3)	3.2 (3.1-3.3)	ND	ND	ND	ND
Percent H_3PO_4 of Aerosol Mass	76	73 (71-75)	76	71.5 (71-72)	75	65.5 (59-72)	69 (48-73)	71 (59-74)	--	--	--	--
Particle Size												
Mass Median Aerodynamic Diameters (μm)[b]	.635 (.63-.64)	.64 (.62-.66)	.885 (.88-.89)	.88 (.82-.94)	--	--	--	--	ND	ND	ND	ND
Respiratory Gases[c]												
O_2 (%)	22	22 (22-22)	22	22 (22-22)	22	19 (18-19)	17 (17-18)	18 (16-21)	21	21 (19-21)	18 (18-19)	18 (18-19)
CO_2 (ppm)[d]	665	877 (847-907)	907	1028 (968-1089)	968	907 (847-968)	726 (726-847)	847 (726-968)	605	677 (629-726)	605 (484-605)	605 (605-629)
CO_2 From Burn (ppm)[d]	108	321 (290-350)	351	472 (411-532)	411	351 (290-532)	169 (169-290)	260 (169-387)	-- --	-- --	-- --	-- --

Table 3 (Continued)

	Target Concentration											
	2.0 mg/1		4.0 mg/1		6.0 mg/1				0.0 mg/1			
Extrusion setting	76 µm		180 µm		293 µm							
Exposures Variable	1	2	1	2	1	2	3	4	1	2	4[f]	4[f]
Contaminant Gases[c]												
CO (ppm)[e]	8.4	12.1 (6-18)	17	22 (7-36)	27	30 (24-36)	24 (22-30)	23 (6-30)	ND	ND	ND	ND
PH_3 (ppm)	6	ND	.06	ND	NC	ND	.12 (ND-.24)	.12 (ND-.12)	ND	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND	ND	7	57 (30-85)	60 (12-121)	67 (ND-121)	ND	ND	ND	ND
Exposure Duration/ Chamber Conditions												
Length of Exposure (min)	75	81 (80-82)	77	85.5 (80-91)	77	86 (84-88)	85 (83-86)	83 (74-84)	77	96 (92-100)	82 (75-94)	77.5 (73-89)
Temperature (C°)	(21-22)	(19-20)	(22-23)	(19-22)	(21-22)	(20-22)	(21-23)	(21-25)	(22-23)	(19-25)	(21-23)	(22-23)
Relative Humidity (%)	54	65 (63-67)	52	63.5 (63-64)	54	58.5 (54-63)	55 (55-65)	55 (50-65)	57	65.5 (65-66)	53 (48-65)	48.5 (46-66)

[a] Median and range statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on these indices. The single exposure data are the actual measurements obtained--not median and range.

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the 293 µm extrusion setting due to the rapid overloading of particles on cascade impactor crystals obscuring the measurements. Small volume sample sizes were insufficient to determine MMAD values for filtered-air exposures (ND = Not Detected).

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was:

$$\text{Actual Tube Value} \times \frac{(760 \text{ mm Hg})}{(628 \text{ mm Hg})}.$$

Table 3 (Continued)

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting.

[e] The EPA standard for CO is 35 ppm maximum for a 1-h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were <30 ppm. This is an unexpected result at the 293 μm extrusion rate because Task 1 data revealed that a rate of 270 μm produced ≥ 30 ppm CO; this result is unexplained (ND = Not Detected).

[f] Two independent, 4-exposure, filtered-air control groups of prairie dogs were used; these were either minimally or regularly handled by research personnel to assess possible effects of strenuous handling upon gross pathology.

The respiratory gases are also within acceptable limits. The O_2 values vary between 16 and 22 percent; whereas, the CO_2 values vary between lows of 605 ppm for certain of the filtered-air groups and highs of 968 and 1,089 ppm for the RP/BR-aerosol groups.

For the contaminant gases of CO, PH_3 and C_6H_{14}, a mixed pattern of results were obtained. First, PH_3 was rarely detected in the chamber atmosphere during the RP/BR aerosol exposures. Only traces of this contaminant occurred. Second, median CO values varied between 8 and 30 ppm for specific groups. Third, relatively high median values of C_6H_{14} were obtained for burns conducted at the 293 µm extrusion pump setting. Median C_6H_{14} detections ranged between 7 and 67 ppm for exposures at this high RP/BR extrusion setting, with some extreme readings of 121 ppm observed. The 1-h Short-term Threshold Limit Value for this contaminant is 50 ppm (National Research Council, 1977). We were unable to account for the cause of these high C_6H_{14} readings; however, it should be noted that Moneyhun et al. (1988) found sizeable differences for C_6H_{14} as measured by industrial-hygiene-type tubes versus gas chromatography/mass spectrophotometry (GC/MS). The GC/MS estimates for exposures at the 293 µm extrusion pump setting equaled about 5 ppm C_6H_{14} (see Moneyhun et al., 1988; Appendix C). No C_6H_{14} contaminant was detected in exposure atmospheres at the 78 or 180 µm extrusion pump settings.

Finally, the exposure duration and chamber conditions were acceptable (see Table 3). Median lengths of exposures varied between 75 and 86 min, with slight differences caused by extra time needed to vent the chamber during some RP/BR burns. Within-chamber temperatures were always between 19 and 25° C for either RP/BR-aerosol or filtered-air exposures. Relative humidity varied within the acceptable limits of 46 to 67 percent for either chamber during all exposure treatments.

b. Mortality and Symptomatology

Mortality.--For the RP/BR aerosol target concentration range of 0.0 to 6.0 mg/l and for up to 4 successive daily 80-min exposures, no deaths occurred in any of the 72 prairie dogs. At the 250 l/min air flow rate maintained in the RP/BR aerosol chamber, the extrusion pump setting of 293 µm produced close to the maximum RP/BR-aerosol chamber concentration that could be achieved with the ORNL extruder. These levels probably exceeded field exposure concentrations and gave a strong indication that prairie dogs can readily survive high aerosol concentrations in multiple exposures normally lethal to laboratory rats.

Symptomatology.--Inter-observer reliability was assessed for 3 investigators (i.e., BEJ, RTS and SAS) each scoring the symptoms shown by 18 animals during the Pre-exposure Phase. Overall, the reliability of reported symptoms by the investigators was viewed as sufficient to detect any overt clinical changes in the condition of the prairie dogs. Independent ratings of the 18 prairie dogs showed 100 percent agreement for the following symptom categories: body posture (resting and post exercise), respiratory congestion (resting and post exercise) and coat condition (groomed versus ungroomed). There was 94.5 percent agreement for the categories of normal vocalizations and affected vocalizations. The poorest agreement among investigators was for the number of animals that attempted any vocalization (87.5 percent agreement) and for those that displayed aggressive responses (81.3 percent agreement).

Symptomatology data from the 2 sub-studies have been combined and are summarized in terms of total positive symptom counts over 3, 7-day periods: Pre-exposure--the 7 days prior to RP/BR-aerosol or filtered-air exposure; post-expsoure 1--the first 7 days after the last RP/BR-aerosol or filtered-air exposure and Post-exposure 2--a second period with data taken for 7 sessions on Days 10, 13, 16, 19, 22, 25 and 28. The total symptom counts reflect the number of prairie dogs within each group showing a given symptom in each 7-session period. That is, regardless of how many times an animal showed a symptom on a given day, a single rating was made for that symptom. These counts have a theoretical range of 0 to 42 (i.e., the number of positive ratings for each of 6 prairie dogs on each of 7 days). Symptom counts of 1 to 4 thus indicate fairly low rating frequencies (<10 percent). The occurrence of some positive symptoms in the Pre-exposure Period also limited the interpretations of RP/BR-aerosol effects.

In general, the incidence of symptoms in each category was quite low for all 12 of the prairie dog groups. Data for the symptom measures, other than body weight and water consumption, are shown in Figures 7, 8 and 9 (see Appendix F for actual frequency counts). Two main symptom effects are evident in these figures: vocalization and respiratory congestion.

Vocalizations generally decreased in frequency for all groups over the Pre-exposure, Post-exposure 1 and Post-exposure 2 Periods. Lost or affected vocalization was a main symptom associated with the 6.0 mg/l target concentration RP/BR aerosol groups. The most severely affected animals were those that received 4 daily exposures at this concentration. When vocalizations were observed for this group, essentially half of the vocalization counts (11:22) were in symptom categories of affected or lost during the Post-exposure 1 Period (see Fig. 9).

Respiratory congestion increased in incidence, particularly post exercise. This effect was again only evident in the 6.0 mg/l target concentration groups, especially those groups given 3 or 4 inhalation exposures at this level (see Fig. 9).

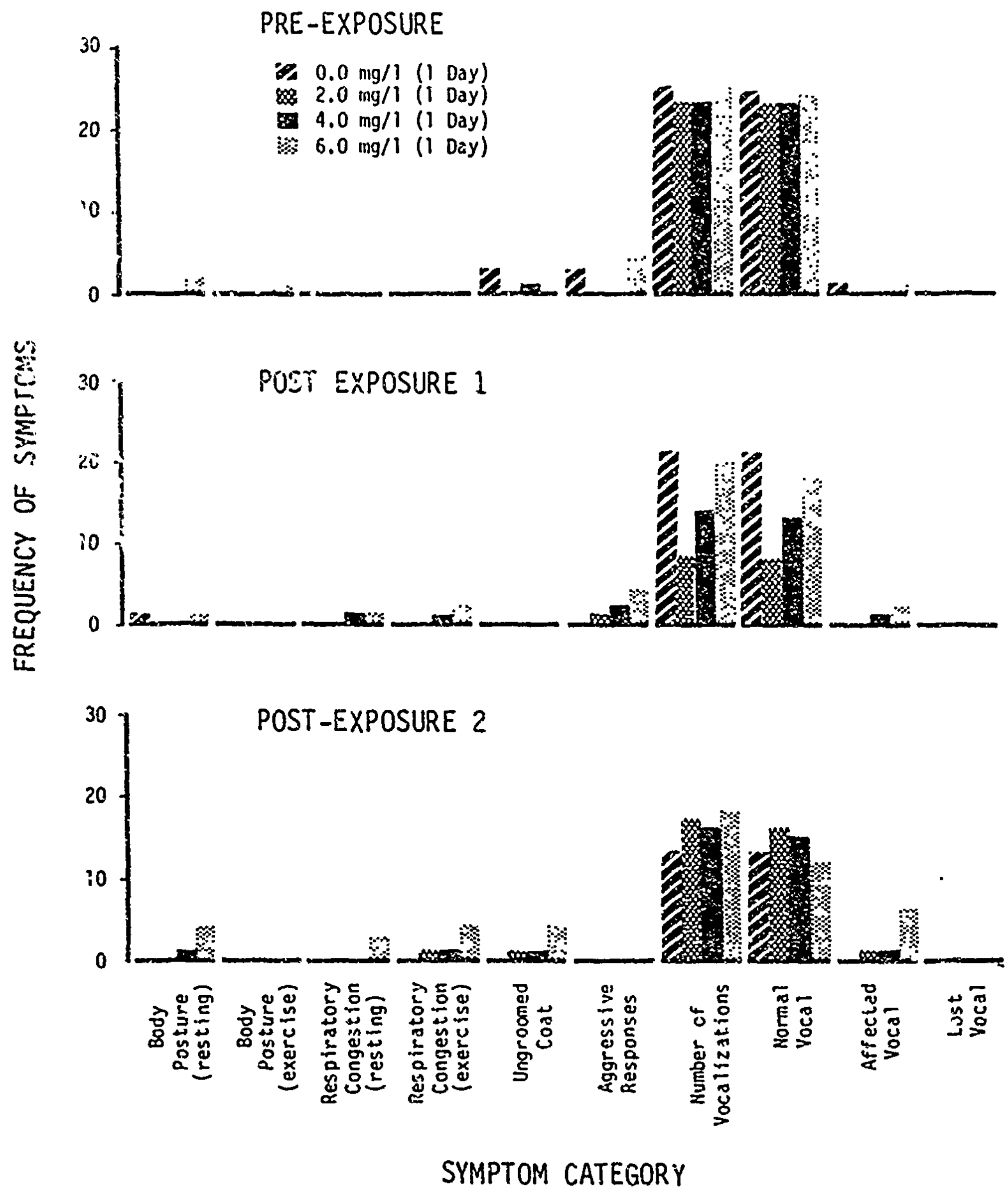

Figure 7. Bar graphs of the Pre-, Post 1 and Post 2, 7-day composite symptom frequencies for prairie dogs treated with 1, 80-min exposure to RP/BR-aerosol target concentrations of 2.0, 4.0 and 6.0 mg/l or filtered air (0.0 mg/l).

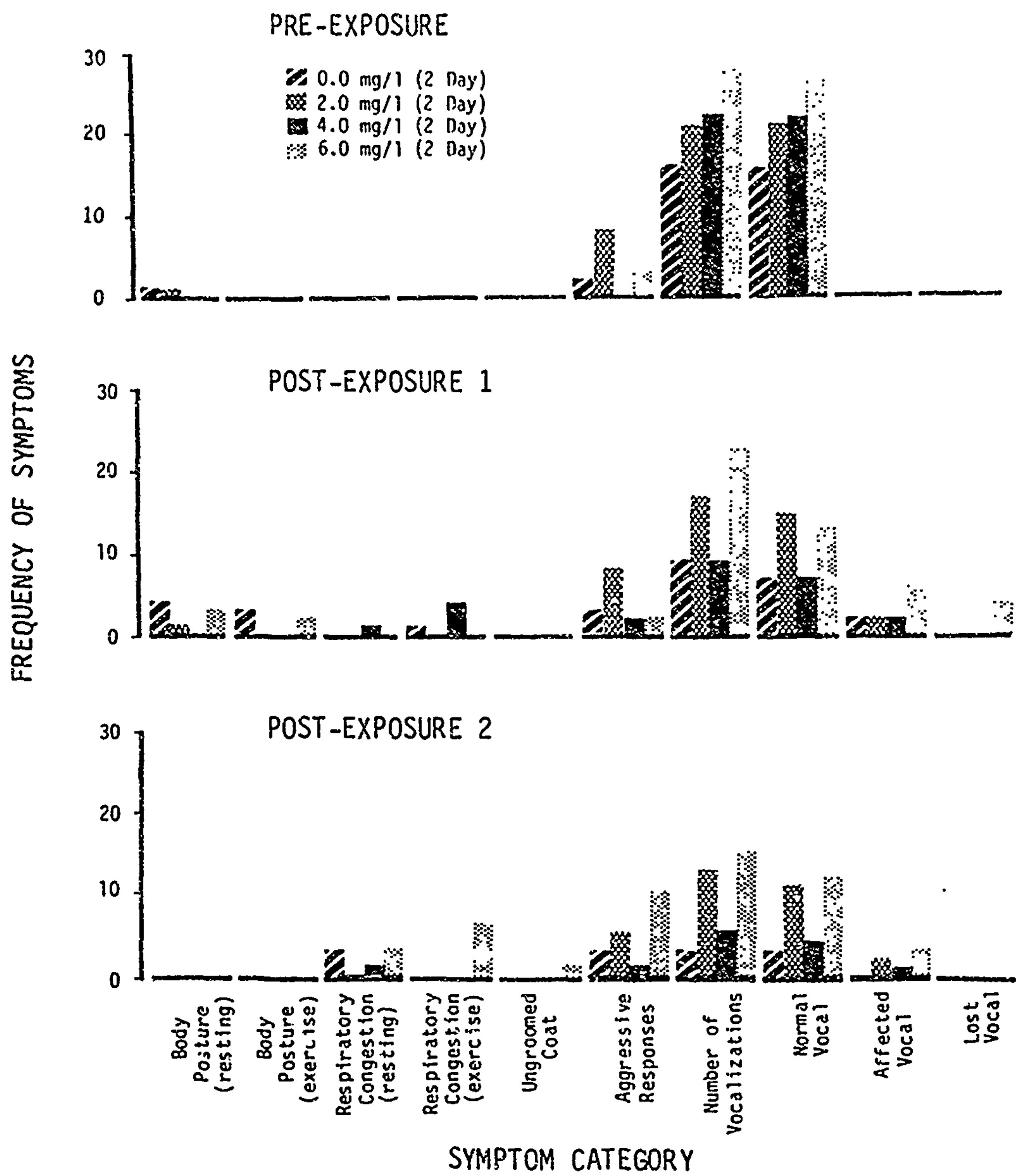

Figure 8. Bar graphs of the Pre-, Post 1 and Post 2, 7-day composite symptom frequencies for prairie dogs treated with 2, 80-min exposures to RP/BR-aerosol target concentrations of 2.0, 4.0 and 6.0 mg/l or filtered air (0.0 mg/l).

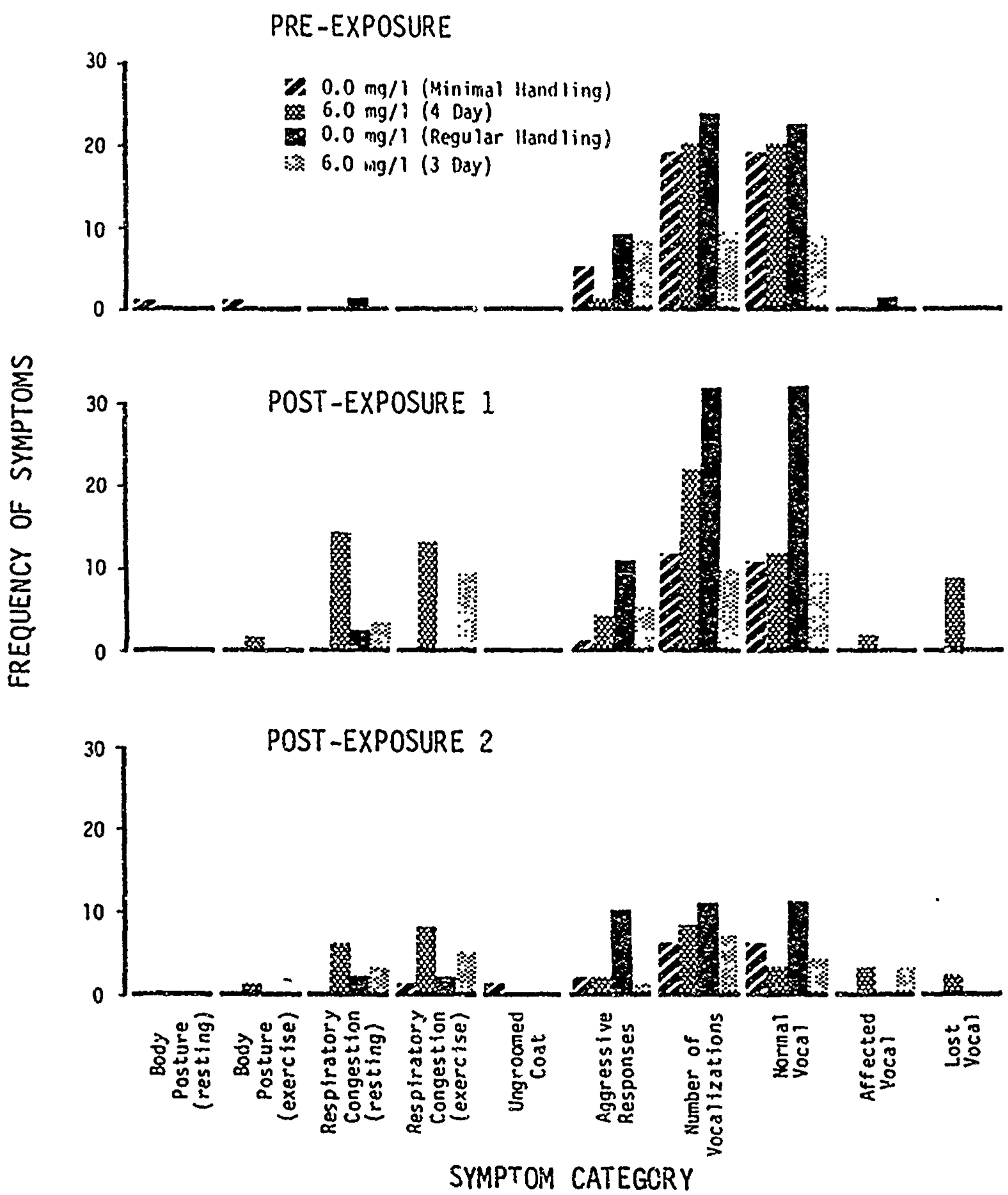

Figure 9. Bar graphs of the Pre-, Post 1 and Post 2, 7-day composite symptom frequencies for prairie dogs treated with 3 and 4, 80-min exposures to RP/BR-aerosol target concentrations of 6.0 mg/l or 4, 80-min exposures of filtered air (0.0 mg/l) with minimal and regular handling.

All other post-exposure symptom frequencies were either not substantially changed from the Pre-exposure Period or were at low levels when compared to filtered-air (0.0 mg/l-control) group frequencies.

<u>Body weight effects</u>.--The body weight data were analyzed for RP/BR aerosol exposure effects using 2 ANOVA designs. Both ANOVAs compared body weights on the last Pre-exposure Day (Day 0) with 14 Post-exposure Sessions (i.e., Days 1-7, 10, 13, 16, 19, 22, 25 and 28). The initial analysis only dealt with the 48 prairie dogs (8 groups) given 1 or 2 exposures to RP/BR aerosol target concentrations of 0.0, 2.0, 4.0 or 6.0 mg/l. As stated, this design was a 4 (Concentration) x 2 (Sex) x 2 (Exposure) x 15 (Session) factorial, with Sessions treated as a repeated measures factor (Winer, 1971).

Three significant effects were obtained in this ANOVA: Exposure x Session ($F = 1.69$, $df = 14/448$, $P < 0.054$), Sex ($F = 7.09$, $df = 1/32$, $P < 0.012$) and Session ($F = 99.48$, $df = 14/448$, $P < 0.0001$).

Results of post hoc Duncan Multiple Range Tests (Waller and Duncan, 1979) among Exposure x Session means indicated that both the 1- and 2-Exposure Groups were significantly different ($P < 0.05$) on the last Pre-exposure Day with the 2-Exposure Group 2.7% heavier, and that this difference remained through Day 10 of Post-exposure. On Days 13, 19, 25 and 28, however, these 2 groups were not significantly different in weight. The 2-Exposure Group was only 1.2% heavier on Day 28. The general trend suggests that prairie dogs in the 1-Exposure Group gained body weight slightly faster than animals in the 2-Exposure Groups after Day 10 Post-exposure. Although animals that received 1 or 2 exposures generally showed a brief pause in weight gain for 1 to 2 days post RP/BR-aerosol or filtered-air exposure, no strong long-lasting body weight suppression effect was evident in these data.

Main effects for Sex and Sessions were expected in this ANOVA. Mean male body weights were 11.2 percent greater than mean female weights (1076.3 g vs 955.7 g, respectively). The Session effect was not analyzed with post hoc Duncan's Tests; however, the general trend was for the combined 48 animals to gain approximately 4.5 g per day throughout the 14 session Post-exposure Period. The mean weight of the 48 animals rose from 963.9 g on Pre-exposure Day to 1090.9 g on the last Post-exposure Day--a 13.6 percent increase across Sessions.

A second ANOVA design was applied to data sets from the 2 sub-studies. It involved body weight data from 36 prairie dogs (6 groups) that were given either 1, 2 or 4 RP/BR aerosol exposures at the 6.0 mg/l target concentration or 1, 2 or 4 filtered-air exposures at the 0.0 mg/l target concentration. The complete factorial design included the factors of 2 (Concentration) x 2 (Sex) x 3 (Exposure) x 15 (Session), with Session treated as a repeated measures factor.

Results of this ANOVA yielded 3 significant main effects: Exposure ($F = 5.39$, $df = 2/30$, $P < 0.01$), Sex ($F = 10.02$, $df = 1/30$, $P < 0.003$) and Session ($F = 62.79$, $df = 14/420$, $P < 0.0001$). None of the interaction terms were significant.

Each of the main effects was somewhat expected. The Exposure effect was an artifact attributed to the 4-Exposure Groups being significantly heavier than the 1- and 2-Exposure Groups at the start of the study. Body weight means for the 1-, 2- and 4-Exposure Groups were 996.2 g, 1062.5 g and 1182.0 g, respectively. The animals in the 4-Exposure (RP/BR aerosol and filtered-air) Groups had been housed in the DWRC quarantine building for approximately 4 months longer than the earlier studied animals.

The Sex effect again confirmed that the male prairie dogs consistently weighed more than the females throughout the study (i.e., means of 1170.9 g vs 1018.7 g, respectively).

The Session main effect was analyzed using Duncan's Multiple Range Tests, and several patterns were indicated. There was a slight pause in body weight gain for 1 day post exposure, and the mean weight on Day 1 Post-exposure was not significantly different from Day 7 of Pre-exposure. Between Day 1 and Day 2 Post-exposure, there was a significant ($P < 0.05$) and rapid increase in body weight that could have indicated recovery from RP/BR-aerosol and/or filtered-air exposure effects. Thereafter on Day 2 through Day 28 Post-exposure, there was a linear gain in mean body weight for all animals. On each successive 3- to 6-day interval during this Post-exposure Period, mean body weight was significantly ($P < 0.05$) increased.

In summary, both ANOVA designs indicated that neither RP/BR-aerosol target concentrations over the range of 2.0 to 6.0 mg/l nor 1 to 4 successive 80-min exposures had significant and sustained effects on prairie dog body weights over 28 days of post exposure. The only indication that repeated RP/BR aerosol exposures may have affected body weight was in the initial ANOVA, as detected by the significant Exposure x Session interaction term. Still, differences in body weight between the 1- and 2-exposure groups across sessions were very slight--2.7 percent on Day 7 Pre-exposure reduced to 1.2 percent on Day 28 Post-exposure. Biologically, these changes are probably inconsequential.

Water consumption effects.--Water consumption data were analyzed using ANOVAs that were essentially similar to those described for the body weight data sets. The first analysis involved the 48 prairie dogs (8 groups) given 1 versus 2 exposures at RP/BR-aerosol or filtered-air target concentrations of 0.0, 2.0, 4.0 or 6.0 mg/l. Factors included in this design were: 4 (Concentration) x 2 (Sex) x 2 (Exposure) x 15 (Session), with the Session variable treated as a repeated measures factor (Winer, 1971).

Five significant effects were found: Concentration x Exposure x Session (F = 2.09, df = 42/444, P < 0.0001), Concentration x Session (F = 2.26, df = 42/444, P < 0.0001), Exposure x Session (F = 2.41, df = 14/444, P < 0.0029), Sex x Session (F = 2.18, df = 14/444, P < 0.0079) and Session (F = 7.64, df = 14/444, P < 0.0001).

In an attempt to analyze the Concentration x Exposure x Session interaction effect, 3 component 2-way interaction graphs were plotted as shown in Figure 10. These graphs indicate the Concentration x Session effect, the Exposure x Session effect and the Concentration x Exposure effect.

As indicated in Figure 10, the Concentration x Session effect (P < 0.0001) shows 2 main trends. First, the 6.0 mg/l RP/BR-aerosol group drank approximately 21 percent more than the 0.0 mg/l filtered-air (control) group on Days 10 through 28 Post-exposure. Second, the 2.0 and 4.0 mg/l RP/BR aerosol groups drank approximately 33 percent less than the 0.0 mg/l filtered-air (control) group animals on Days 22 through 28 Post-exposure. Thus, RP/BR-smoke inhalation appears to produce divergent water consumption behavior depending upon the aerosol concentration.

The Exposure x Session effect (P < 0.0029) also indicated 2 main trends. First, the 1-Exposure Group animals drank less than their pre-exposure level for up to 7 days post exposure, but the 2-Exposure Group animals were suppressed in drinking for only 3 days post exposure. Second, although the 1-Exposure Group displayed consistently lower water consumption through Day 25, the 1- and 2-Exposure Groups reversed consumption levels on Day 28.

Finally, the Concentration x Exposure effect (P < 0.22; non-significant) indicated that for both 0.0 and 4.0 mg/l groups, the 2-Exposure Group animals drank nore than the 1-Exposure Group animals. The reverse occurred for the 2.0 and 6.0 mg/l Groups--the 2-Exposure Groups drank less than the 1-Exposure Groups. This effect is probably nonspecific to the treatments. Rather, extreme variability in water consumption levels for these means over 15 sessions negated significant differences.

Thus, the 3-way (Concentration x Exposure x Session) term appears to reflect mainly the operation of 2, 2-way interactions: Concentration x Session and Exposure x Session. The major component of this complex interaction is attributed to late post exposure effects from the 3 RP/BR-aerosol concentrations. RP/BR-aerosol did not generally suppress water consumption over the 28-day Post-exposure Period. Instead, one of the clearest results was a 21 percent increase in water consumption by the 6.0 mg/l Exposed Group late in the Post-exposure Period. Multiple exposures of prairie dogs to RP/BR-aerosol concentrations of 6.0 mg/l produced elevated water intake between 10-28 days following inhalation. Whether this could be explained by water loss through

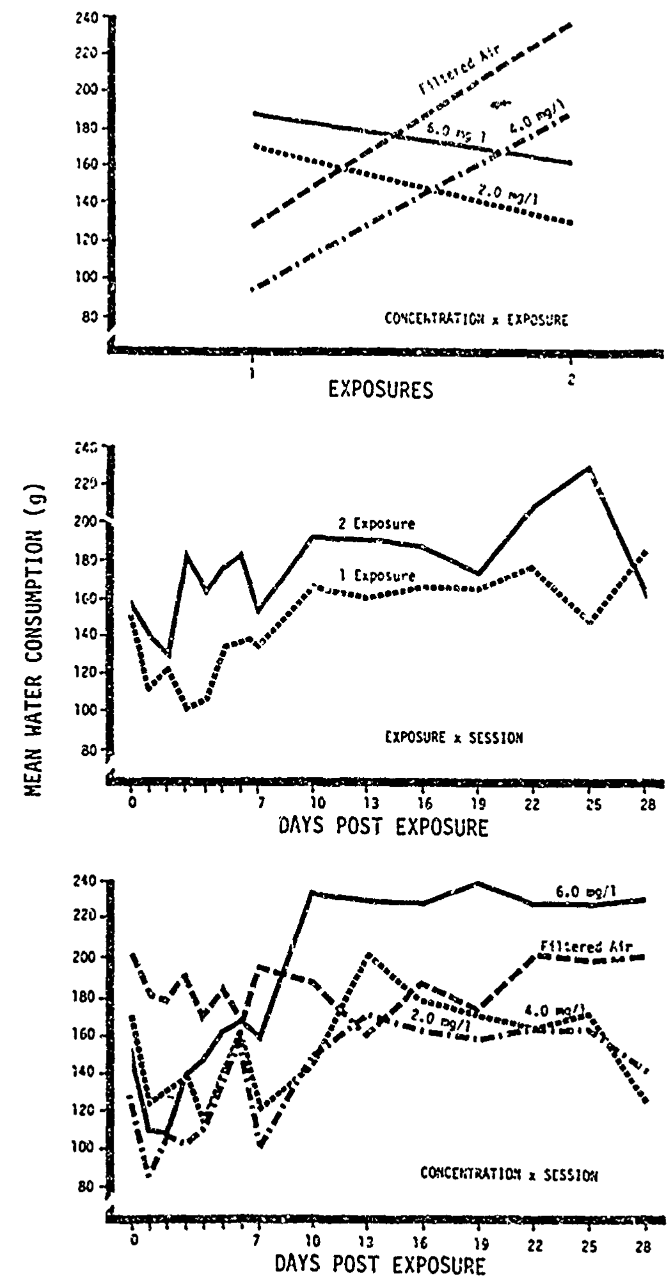

Figure 10. Upper panel. Mean water consumption levels of prairie dog groups exposed to 0.0, 2.0, 4.0 or 6.0 mg/l target concentrations of RP/BR aerosol for 1 vs. 2, 80-min exposure sessions. Data for all sessions have been pooled.

Middle panel. Mean water consumption levels of prairie dog groups that received 1 vs. 2, 80-min exposures. The 3.0 and 6.0 mg/l target concentration data have been pooled.

Bottom panel. Mean water consumption levels of prairie dog groups that received 0.0, 2.0, 4.0 or 6.0 mg/l target concentrations of RP/BR aerosol. The 1- and 2-exposure data have been pooled.

pulmonary edema, elevated body temperature, hyperactivity or other factors could not be determined, but these variables would be likely candidates for evaluation in future studies.

Another significant interaction effect, which was independent of the above described 3-way term, was the Sex x Session effect. Duncan's Multiple Range Test on these means revealed that male and female prairie dogs were not significantly different on the last Pre-exposure day (Day 0) and throughout most of the Post-exposure Period (Days 1-19). On Days 22 and 28, however, the males drank significantly more than did the females. Males showed approximately 25 percent more water intake than did the females during the last week of post exposure. One interpretation of this effect is that males were more debilitated by RP/BR-aerosol exposure and developed edema; whereas, females were less affected and showed enhanced recovery from RP/BR-aerosol exposure. Of course, without additional functional studies of the sex difference, these data trends also remain unexplained.

Taken together, the significant effects in this ANOVA indicate several trends. The 6.0 mg/l exposure groups drank much greater amounts of water late in the Post-exposure Period. The 2.0 mg/l and the 4.0 mg/l exposure groups drank less than did control group (0.0 mg/l) animals after Day 19 of post exposure. Males tended to drink more water late in the Post-exposure Period, and females reduced their water intake during this same period.

A second 4-factor ANOVA was run on the combined data set for groups given 1, 2 or 4 RP/BR aerosol exposures at either the 0.0 mg/l (filtered-air) or at 6.0 mg/l (RP/BR aerosol) target concentration levels. The design involved the following factors: 2 (Concentration) x 2 (Sex) x 3 (Exposure) x 15 (Session), with Session treated as a repeated measures factor.

Three significant terms were found: Exposure x Session ($F = 2.70$, $df = 28/417$, $P < 0.0001$), Concentration x Session ($F = 4.25$, $df = 14/417$, $P < 0.0001$), and Session ($F = 8.03$, $df = 14/417$, $P < 0.0001$).

The Exposure x Sessions effect, when further analyzed graphically along with Duncan's Tests, indicated a general trend toward more suppression of water intake in the 4-Exposure Group (compared to the 1- and 2-Exposure Groups) late in the Post-exposure Period. For Days 10-28, 6 of 7 of the means for the 4-Exposure Group were lower than were the means of either the 1- or 2-Exposure Groups (or both Groups). The degree of initial suppression of drinking immediately after exposure (i.e., Day 1) could not be assessed due to a significant ($P < 0.05$) difference between the 2- versus 4-Exposure Groups on the pre-exposure day (i.e., Day 0).

The Concentration x Session term gave a clear confirmation again that there was significantly less water consumption by the 6.0

mg/l RP/BR-aerosol-exposed Group animals initially after exposure, and then significantly more water was consumed (compared to the 0.0 mg/l Filtered-air-exposed Group animals) late in the Post-exposure Period.

Figure 11 shows this Concentration x Session interaction effect. As depicted, the 2 groups do not differ statistically on the pre-exposure day (Day 0). Immediately after exposure, however, the 6.0 mg/l Groups showed an initial drop (approximately 37 percent; $P < 0.05$), and then they rapidly increased their water intake to normal levels through Day 7 Post-exposure. On Day 10, and on Days 19-28, however, the 6.0 mg/l Group animals drank significantly more water (approximately 31 percent; $P < 0.05$) than did the 0.0 mg/l Filtered-air Group. This increased water intake by the 6.0 mg/l Group was also in close agreement with the results of the first ANOVA (partially on the same data set) where 4 target concentrations (0.0, 2.0, 4.0 and 6.0 mg/l) were compared at 1- versus 2-exposures.

c. Necropsies

Post mortem examinations were performed on a total of 72 animals 30 days after the last RP/BR-aerosol or filtered-air exposures. Veterinarians performing the necropsies were only told which animals had received filtered-air exposures. Practice in identifying the appearance of normal organs and tissues was thought to be the best course of action to enhance the veterinarians' familiarization with gross necropsy of this species. Observed incidence of abnormalities in the 10 organs examined by RP/BR-aerosol or Filtered-air Group are listed in Table 4 along with a coded description of specific pathology. The causes of many observed conditions, particularly for lung hemorrhages, darkened/enlarged/congested livers and abnormal coloration of the spleens, are currently unexplained. These abnormalities occurred in both the filtered-air-exposed animals as well as in the RP/BR aerosol-exposed animals. The only indication of some pathology possibly being due to the RP/BR aerosol treatments was an increase in the incidence of more severe congestion observed in the lungs of the 6.0 mg/l Groups. This increased incidence was, however, quite small when the 24 filtered-air exposed animals were compared to the 24 animals previously exposed to 6.0 mg/l RP/BR aerosol (i.e., 12.5 percent versus 20.8 percent, respectively).

Thus, the necropsy examinations yielded no strong consistent pattern. This lack of pathological effects was consistent with the findings of Aranyi (1983b). Burton et al. (1982) did report laryngeal and epiglottal injuries in albino rats, but much higher concentrations of RP/BR aerosol were achieved during their exposure periods.

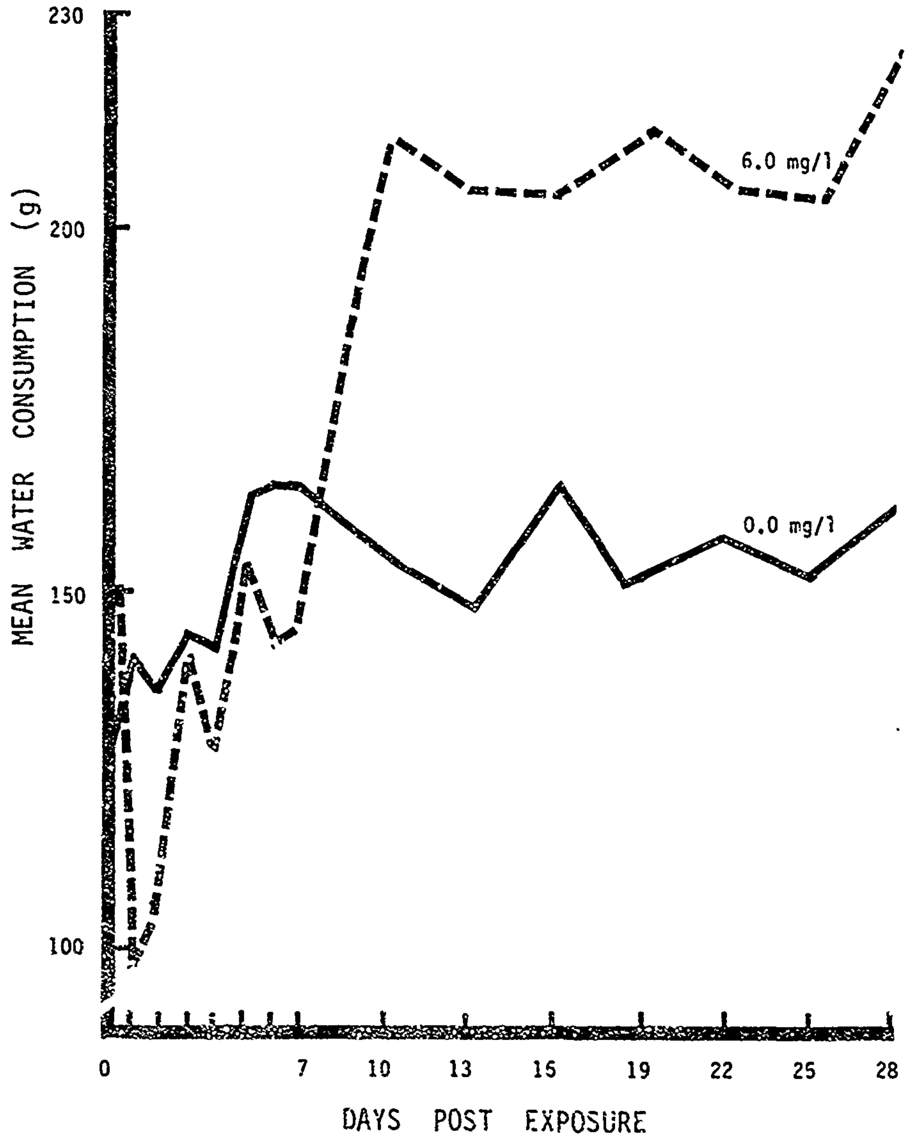

Figure 11. Mean water consumption by prairie dogs as a function of time after exposure for the 0.0 vs. 6.0 mg/l RP/BR smoke exposure levels. The 1, 2, and 4 daily smoke exposure groups have been combined (n = 18/group) to indicate the two-way interaction effect. Day 0 is the last pre-exposure day and Days 1-28 are the mean water intake values on the days following the last daily exposure to RP/BR smoke.

Table 4. Prairie dog necropsy data. Incidence of each observed type of abnormality has been listed for the 12 groups (n = 6 animals/ea).[a]

Target	Filtered-air Exposed				RPBR-aerosol Exposed							
Concentration (mg/l)	0.0	0.0	0.0	0.0[b]	2.0	2.0	4.0	4.0	6.0	6.0	6.0	6.0
Number of exposures	1	2	4	4	1	2	1	2	1	2	3	4
Nasal passages				1H			1EF				1H, 1L	
Trachea				1H							1DD	
Larynx				1EF								
Epiglottis												
Bronchi				1H		1H						
Lungs	2H	3H	2C	1C++	2H, 3L		3H	3H	4H	1H, 1C+++	1C, 1C+	1C+,1H 1C++
Heart		1S+		1S+++	1S++, 1S+++					1S+		
Liver	1DL	1S+	1MC	6C, 2DL	1DR		1S+, 2S++	2DD, 1MC		1S+, 1DL		2C+
Spleen	1L, 1DD, 1WN	5D, 1WN		1H, 1WN	1S+, 1DM			2DL, 1DD, 1WN	1DD, 1DG	1S+, 1DD, 1WN		
Kidneys				1DL								1C+, 1S-

Table 4 (Continued)

Key:

EF	= excess fluid/phlegm/mucus	S+++	= enlarged--highly
C	= congested--slight	D	= discolored
C+	= congested--moderate	DL	= light/pale color
C++	= congested--heavily	DD	= darkened color
C+++	= pneumonia	DR	= reddish color
H	= hemorrhages	DG	= greenish color
L	= lesions	DM	= metabolic sheen
S-	= reduced size	MC	= mottled color/abscesses
S+	= enlarged	WN	= white nodules/nodules
S++	= enlarged--moderately		

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[a] Descriptions of gross abnormalities have been summarized from the Necropsy Data Sheets (e.g., see Appendix D).

[b] These filtered-air (Control) group prairie dogs were not physically restrained for respiratory congestion evaluations via stethoscope. This was to ensure that these animals would have minimal pathology induced by this potential means.

d. Histopathology

Table 5 summarizes the histopathology observed for lung, liver, trachea, larynx and nasal turbinates specimens of the 72 prairie dogs assigned to the RP/BR-aerosol and Filtered-air Groups. Appendix D contains copies of the NVSL Reports from which this table was derived.

Histological examinations of the prairie dog organ tissues yielded a mixed, equivocal pattern of results. The lack of standard histology slides for this species, coupled with the pathologists' admitted unfamiliarity with potential injury caused by inhalation of acid aerosols, contributed to these results.

To compare overall differences between RP/BR-aerosol and filtered-air conditions, we pooled the frequencies of certain descriptors for specimens in the 8 RP/BR-aerosol and 4 Filtered-air Groups. A number of histopathological descriptors (see Table 5) yielded roughly equivalent percentages of occurrence between animals in these pooled groups. This suggested that much of the observed histopathology was non-specific to RP/BR-aerosol exposure.

The percentage incidence of "interstitial pneumonia" (IP) for lung tissue was 15 versus 13 for RP/BR and for filtered-air animals, respectively. This finding indicates that IP is naturally present in about 10 to 15 percent of prairie dogs, and that RP/BR aerosol caused no increased incidence, *per se*.

For liver specimens, incidences of "mild, moderate, or severe, diffuse hepatocellular swelling and degeneration with cholestasis" (MC, C or SC) were 98 and 88 percent for RP/BR-aerosol and filtered-air animals, respectively. "Minimal to mild, multifocal, hepatocyte necrosis" (MU or U) was found in 10 versus 4 percent, respectively, of animals in these conditions. A wide disparity in certain histological lesions of liver tissues was also reported, but these were non-specific to RP/BR-aerosol exposure.

The percentage of "mild or moderate multifocal, epithelial ulcerations and necrosis" (MU or U) for trachial specimens was 58 versus 54 between these same pools of prairie dogs. Again, these results are indicative of no difference in the histopathology of trachial tissues as a result of RP/BR aerosol inhalation.

The percentage incidence of "mild, moderate or severe multifocal hemorrhage and necrosis" (MH, H or SH) in lung tissues was 87.5 versus 62.5 between "pooled" RP/BR- and filtered-air specimens, respectively. Additionally, severe hemorrhage (SH) occurred in 23 percent of lung tissues for RP/BR-aerosol animals as compared to 8 percent of lung samples from control animals. Although these differences are noteworthy, there is still no clear evidence that any of these hemorrhages resulted from RP/BR-aerosol exposure. Small exertive movements or the position of prairie dogs

Table 5. Frequencies of tissue classifications observed by NVSL pathologists for tissue specimens obtained from prairie dogs administered single or multiple RP/BR-aerosol and filtered-air exposures.[a]

RP/BR Target Concentration	2.0 mg/l		4.0 mg/l		6.0 mg/l				0.0 mg/l (Filtered-air)			
Extrusion Setting	78 μm		180 μm		293 μm							
Exposures	1	2	1	2	1	2	3	4	1	2	4R[b]	4M[b]
Tissue												
Lung[c]			1 NL			1 NL	1 NL		4 NL			1 NL
	1 MH		4 MH	2 MH	1 MH		2 MH	1 MH		2 MH	2 MH	2 MH
	3 H	5 H		2 H	1 H	2 H	3 H	4 H	1 H	3 H	3 H	
	2 SH	1 SH	1 SH	2 SH	3 SH	1 SH		1 SH		1 SH		1 SH
						1 PBLH	1 PBLH	1 PBLH	1 PBLH	1 PBLH	2 PBLH	
	2 IP			2 IP	1 IP		2 IP		1 IP			2 IP
									1 FBG			
					1 PG			1 PG				2 PG
							1 PLG				(1 NE)	
Liver[c]	2 MC				2 MC		1 MC		1 MC	1 MC		
	2 C	2 C	4 C	2 C	2 C	3 C	2 C	3 C	2 C	3 C	3 C	4 C
	2 SC	4 SC	2 SC	4 SC	2 SC	3 SC	3 SC	3 SC	3 SC	2 SC	2 SC	2 SC
	2 N		1 N		1 N	1 N			1 N			
											(1 NE)	
Trachea[c]	3 NL	1 NL	1 NL	1 NL	3 NL	1 NL	1 NL		2 NL	2 NL	1 NL	3 NL
	3 MU	5 MU	3 MU	5 MU	3 MU	3 MU	3 MU	3 MU	3 MU	4 MU	5 MU	
									1 U			
			(2 NE)			(2 NE)	(2 NE)	(3NE)				(3 NE)

Table 5 (Continued)

RP/BR Target Concentration	2.0 mg/l		4.0 mg/l		6.0 mg/l				0.0 mg/l (Filtered-air)			
Extrusion Setting	78 μm		180 μm		293 μm							
Exposures	1	2	1	2	1	2	3	4	1	2	4R[b]	4M[b]
Larynx[c]	2 NL 4 GN	1 NL 4 GN 1 IB	3 NL 3 GN 1 IB	3 NL 1 GN	1 NL 4 GN 1 IB	1 NL 5 GN	1 NL 5 GN 1 IB	1 NL 5 GN	1 NL 5 GN 1 IB	1 NL 5 GN 2 IB	1 NL 5 GN 1 LB	2 NL 4 GN 1 IB
				(2 NE),								
Nasal Turbinates[c]	4 NL 1 S 7 H-NT	5 NL 1 S	5 NL 1 S	4 NL 2 S	5 NL 1 S	6 NL	6 NL	5 NL 1 S	5 NL 1 S	5 NL 1 S	2 NL 4 S	2 NL 3 S

[a] All specimens were examined by APHIS Pathobiology Laboratory, NVSL, Ames, IA. Epiglottal tissues were not included in these examinations. Multiple histopathological descriptors were used with each specimen; hence, descriptor codes exceed numbers of animals for certain tissue by exposure conditions.

[b] The 4 R and 4 M descriptors for the filtered-air groups refer to 4 exposures with regular handling and 4 exposures with minimal handling of prairie dogs, respectively, throughout the range-finding study; minimal handling was instituted to allow assessments of possible gross pathology or histopathology induced by the restraint procedures required during symptom examinations.

Table 5 (Continued)

[c] Key to histopathology:

NL - No lesion seen
MH - Mild multifocal hemorrhage and necrosis
H - Moderate multifocal hemorrhage and necrosis
SH - Severe multifocal hemorrhage and necrosis
PBLH - Peribronchial lymphoid hyperplasia
IP - Interstitial pneumonia
FBG - Foreign body granuloma
PG - Pulmonary granulomas (unknown cause)
PLG - Pulmonary lipogranuloma
NE - Specimen not examined
MC - Mild, diffuse, hepatocellular swelling and degeneration with cholestasis
C - Moderate, diffuse, hepatocellular swelling and degeneration with cholestasis
SC - Severe, diffuse, hepatocellular swelling and degeneration with cholestasis
N - Minimal to mild, multifocal, hepatocyte necrosis
MU - Mild, multifocal, epithelial ulceration and necrosis
U - Moderate, multifocal, epithelial ulceration and necrosis
IB - Intracytoplasmic inclusion bodies in ganglion cells
GN - Lymphocytic ganglioneuritis
H-NT - Hemorrhage in nasal turbinate
S - Sarcocystosis in skeletal muscle

immediately preceding death could have easily caused this effect. In short, MH, H or SH categories are again non-specific for RP/BR-aerosol exposure. This concurs with previous studies that have rarely reported lung changes due to RP/BR in rats (Aranyi, 1985b; Burton et al., 1982).

As stated in the actual Laboratory Report (see Appendix D), "a unique lesion was the presence of intracytoplasmic inclusions, within ganglionic cells of the larynx, associated with ganglioneuritis in several animals." Although these inclusions suggest a viral infection, electron microscopic examination of the specimens revealed no viral particles. Additionally, the incidence pattern of "intracytoplasmic inclusion bodies in ganglion cells" (18) were evenly distributed between RP/BR and control animals (i.e., 64% versus 79%, respectively). Still, NVSL pathologists considered this a specific lesion for this study--an observation worthy of detailed attention in any future research.

D. Toxicity Effects in Rock Doves

1. Procedures

a. Treatment Assignment

Forty-eight rock doves (24 of each sex) were randomly selected from the group of 122 captured doves. A cloacal examination procedure previously described by Miller and Wagner (1955) was used for determining the sex of each dove. Further verification of sex was based upon gross necropsy at the end of the study.

Body weight ranges of 8 groups, each composed of 3 males and 3 females, were approximately matched by pre-assignment ranking of body weights. This first involved rank-ordering the 24 rock doves of each sex by body weight. Three weight classes were arbitrarily developed for each sex by assigning the 8 lowest weight rock doves to the "light" group, the next 8 to the "medium" group, and the heaviest to the "heavy" group. Light weight males ranged from 270 to 329 g, medium weight males ranged from 333 to 352 g, and heavy weight males ranged from 352 to 389 g. For females, these respective ranges were 277 to 307 g, 313 to 334 g, and 335 to 363 g. Next, 1 male and 1 female rock dove from each of the light, medium and heavy weight groups (i.e., 3 males and 3 females) were randomly assigned to 1 of 8 treatment groups.

Six of the 8 groups were to receive either 1, 2 or 3 successive daily exposures to RP/BR-aerosol target concentrations of 3.0 and 6.0 mg/l (i.e, extruder pump settings of 133 and 293 µm). The remaining 2 groups were to receive 4 successive daily exposures to either RP/BR aerosol at the 6.0 mg/l target concentration or to filtered-air at the 0.0 mg/l target concentration.

All exposure sessions were approximately 80 min with minor time differences due to venting of the inhalation chamber. Durations of the 4 filtered-air exposure sessions were matched to the 4-exposure, 6.0 mg/l target concentration group session times.

b. Assessment Paradigm

The rock dove paradigm was comprised of essentially the same sequence of phases as described in the prairie dog sub-studies. A 7-day Pre-exposure (Baseline) Phase was followed by a 1- to 4-day Exposure and a 28-day Post-exposure Phase. Again, 3 sets of toxicity-assessment variables were measured at specific times within the 3 phases: Symptomatology/Mortality, Gross Necropsy and Histology. Specifically, mortality was continually assessed daily throughout all phases. Symptomatology was measured daily during Pre-exposure and Exposure Phases, and then on Days 1-7 (Post 1) and on Days 10, 13, 16, 19, 22, 25 and 28 (Post 2) of the Post-exposure Phase. All rock doves were then euthanized via i.p. injection of sodium pentobarbital 31 days post-exposure. Gross necropsy examinations of 10 organs were conducted immediately post mortem, and sections of 7 of the organs were taken for later histological examinations.

c. Symptomatology/Mortality

A list of 8 symptomatology/mortality categories and their respective operational definitions or procedures for evaluation of RP/BR-aerosol effects in rock doves is shown in Table 6. Qualitative measures were taken for the categories of: body posture, respiratory congestion, plumage condition, aggression, and vocalization. Quantitative measures were taken for the categories of: water consumption, body weight and mortality.

Mortality was determined daily throughout all phases of the range-finding study. An investigator checked each rock dove daily between 0800 and 0900 h MST, prior to symptomatology examinations.

Symptomatology measures were taken on the individual rock doves in each group on the Pre-exposure, Exposure and Post-exposure (Post 1 and Post 2) Phases. Symptom examinations required 4 to 6 min per animal and consisted of the following sequence of events: (a) a visual inspection and determination of body posture and plumage condition with each rock dove in the home cage, (b) removal of the dove from the home cage and determination of an aggression rating and a respiratory congestion rating (rest), (c) placement of the rock dove into a large, ventilated metal container and measurement of body weight, (d) placement of the dove on a perch in the flight cage and inducement of 6 flight crossings of the length of this cage, (e) removal of the dove from the flight cage with an immediate determination of post-exercise respiratory congestion and aggression, and (f) placement

Table 6. List of the 8 symptomatology/mortality categories, plus the operational procedures/definitions, used to rate each rock dove during the 7-day Pre-exposure, 1- to 4-day Exposure and 14-session Post-exposure Phase (i.e, Days 1-7, 10, 13, 16, 19, 22, 25 and 28).

Symptom Category	Operational Procedure/Definition
Qualitative	
Body Posture (rest & post-exercise)	The body posture of each rock dove while at rest in their home cage and after 60 sec of forced flight were recorded. For exercise, each dove was prodded with a 1-m wooden dowel rod to make 6 flights of approximately 3.3 m from perch-to-perch within a 3.3 x 1.6 x 2.6 m fiberglass and wire mesh (1.3 cm^2) flight cage. Abnormal ratings were assigned to birds that were listing forward with head and chest "hunched over."
Respiratory Congestion (rest & post-exercise)	Each dove was checked for signs of respiratory congestion before and after exercise. Abnormal ratings were assigned to birds which displayed "parted (open) beak" or audible breathing sounds of gasping, rasping, wheezing or sneezing. Audible respiratory sounds were determined by holding the dove's head and chest within a few cm of the investigator's ear; a stethoscope was not used for this measurement.
Plumage Condition	The plumage condition of each dove was rated as kempt and unkempt. Unkempt ratings were assigned to feathers which were ruffled or dull in appearance.
Aggression	The aggression that each rock dove displayed toward handlers was scored as high, medium or low. Positive ratings were assigned to doves that persisted in batting the handler's hand with the wings or pecking at the handler when removed from the home cage and examined.
Vocalization (Occurrence & Quality)	The occurrence (present, absent) and quality of vocalizations (normal, affected or lost) were noted for those birds that cooed during the examination session. An occurrence of vocalization was recorded as present if an individual dove was observed emitting sounds during an examination session. Ratings of quality were assigned as follows: Normal--a low pitch (guttural) cooing sound, Affected--a broken or raspy cooing sound and Lost--beak movements indicating unsuccessful attempts to coo, with only the sound of rushing air or gasping emitted.
Quantitative	
Water Consumption (24 h)	The daily water consumption of each dove was measured as the difference in ml of water removed from respective drinker tubes during the measurement period. Time of water availability was also recorded to the nearest min. The intake of each dove was measured using a ruled-ml scale. The waterline (miniscus) was marked with a small rubberband after each tube was filled and affixed to the respective cage; then after each measurement session, a ruled scale was held next to each tube and the difference (ml) between the rubber band and the new waterline (miniscus) determined. Tubes were measured, removed, rinsed, refilled, reattached and banded at the end of each examination session. Differences in the time of water availability were adjusted for a 24-h base using the formula: $\text{24 h Consumption} = \frac{\text{24 (Measured water drunk in ml)}}{\text{(Duration of availability in h)}}$.
Body Weight	The daily or session (Post exposure) body weight of each rock dove was recorded (nearest g) using an electronic balance (Model PE 3600; Mettler Corp., Hightstown, NJ).
Mortality	Death (i.e., lack of respiration and heartbeat) was determined daily for each rock dove during an initial check of all cages by an investigator (i.e., 0800-0900 h MST).

of the dove back into its home cage, with a determination of post-exercise body posture effects. Water consumption levels were measured for all groups at the end of the symptom evaluation period.

d. Gross Necropsy

On Day 31 post exposure, all doves were euthanized with Beuthanasia-D Special solution (sodium pentobarbital) injected i.p. for post mortem analyses. The gross necropsy procedures essentially paralleled those described for the prairie dog tests. The following organs were systematically examined: nasal passages, trachea, larynx, epiglottis, bronchi, lungs, heart, liver, kidneys and spleen (see Appendix D - Standard Gross Necropsy Form).

e. Histological Examinations

Procedures were essentially the same as those described for prairie dogs--identities of specimens were not "blinded" from the pathologists. Sections of the nasal turbinates, lungs and liver, plus the entire trachea, larynx and epiglottis, from each rock dove were preserved together in a specimen jar of 10 percent formalin solution. Again, histological examinations were performed by staff of the Pathobiology Laboratory, Parasitology and Clinical Pathology Section, NVSL. A copy of the histology report for rock doves is presented in Appendix E.

f. Designs and Data Analyses

Symptomatology, mortality, gross necropsy and histopathology data were analyzed using descriptive statistics and graphical illustrations. Pre-exposure baseline data were used for comparing changes in frequencies of the abnormal (positive) symptoms. Symptomatology data were presented in tabular form to indicate changes in the percentage of observations for each measure that were positive, based upon all surviving rock doves.

Water consumption and body weight data were analyzed using two repeated measures ANOVAs (Winer, 1971). Data for the last daily session of the Pre-exposure Phase were compared with the data for 14 daily sessions of the Post-exposure Phase. Due to the death of some doves, the ANOVAs were computed using the General Linear Hypothesis Model (GLM) of the SAS package of programs (SAS Institute, Inc., 1985) and Type III sums of squares.

The first ANOVA was designed to determine whether a higher concentration or a greater number of daily RP/BR-aerosol exposures would cause the greatest change in water intake and/or in body weight loss. Daily water consumption and body weight data were each analyzed with a 4-factor design involving: 2 (3.0

and 6.0 mg/l Concentrations) x 3 (1, 2 or 3 Exposures) x 2 (Sexes) x 15 (Sessions), with Sessions treated as a repeated factor (Winer, 1971).

The second set of ANOVAs was a further test of whether RP/BR-aerosol would cause a change in water intake and/or a decline in body weight after 4 daily exposures when the 6.0 mg/l target concentration group was compared with the 0.0 mg/l (filtered-air) group. The two measures were each analyzed using a 3-factor design involving: 2 (0.0 vs 6.0 mg/l Concentrations) x 2 (Sexes) x 15 (Sessions), with repeated measures on Sessions.

Where significant effects were found in each ANOVA, post hoc Duncan Multiple Range Tests (Waller and Duncan, 1969) were used for pair-wise comparison of all means.

Gross necropsy and histological data were treated descriptively. That is, the percentages of specimens found to have clinical abnormalities were computed for RP/BR-aerosol and for filtered-air exposure groups and were then compared.

2. Results and Discussion

a. Aerosol and Filtered Air Measurements

Table 7 presents medians and ranges of variables that characterized the RP/BR-aerosol or filtered-air exposure conditions. As shown, the initial 7 columns of Table 7 refer to the separate groups of rock doves administered between 1 to 3 or 1 to 4 successive daily exposures of RP/BR-aerosol at the 133 µm or 293 µm extrusion pump settings (i.e., 3.0 or 6.0 mg/l target concentrations, respectively). One control group involving 4 successive daily filtered-air exposures was used in this study.

Examination of the aerosol mass statistics in Table 7 reveals that median values varied from 198.7 to 209.9 mg for the 73 to 81 min burns at the 133 µm extrusion pump setting. The median H_3PO_4 levels varied between 140.1 and 159.1 mg--71 to 76 percent of the aerosol mass. As with the prairie dog exposures, these data are consistent with prior data at equivalent extrusion pump settings (Sterner et al., 1988; Moneyhun et al., 1988), considering the longer filter-collection period involved.

Again, MMAD values concur with Sterner et al. (1988). Median aerosol particle sizes varied from .79 to .825 µm for the burns conducted at the 133 µm extrusion pump setting, particles within the respirable range (Phalen, 1984).

Chamber air quality was acceptable, with O_2 values remaining essentially constant at 19 percent throughout all conditions. The CO_2 values were very acceptable and varied between 575 and

Table 7. Median (minimum-maximum) statistics of the aerosol and air quality measurements characterizing the single or multiple RP/BR and filtered-air (control) exposures conducted at the 133 and 293 μm extrusion settings for the toxicity range-finding studies with rock doves in Task 2.[a]

	TARGET CONCENTRATION							
	3.0 mg/l			6.0 mg/l				0.0 mg/l
Extrusion setting	133 μm			293 μm				
Exposures	1	2	3	1	2	3	4	4
Variable								
Aerosol								
Aerosol Mass (mg)	209.9	198.7 (190.5-206.9)	200.4 (175.9-203.4)	387.8	347.1 (334.1-360.1)	353.3 (338.2-385.7)	357.8 (320.3-386.1)	.1 (-4.1-.7)
Aerosol Mass Concentration (mg/l) 80-min	2.9	2.5 (2.2-2.7)	2.8 (2.5-2.8)	5.0	4.5 (4.3-4.7)	4.9 (4.3-5.0)	4.6 (4.0-4.9)	.008 (.001-.05)
Steady-state Concentration (mg/l)	3.3	3.3 (3.1-3.4)	3.2 (2.9-3.3)	6.3	5.6 (5.3-5.9)	6.2 (5.9-6.4)	6.1 (5.7-6.4)	--
H_3PO_4 Titration (mg)	159.1	140.1 (132.2-148.0)	141.6 (131.9-150.6)	280.5	264.8 (249.6-280.5)	251.5 (249.2-272.2)	254.8 (232.4-272.9)	0.0
H_3PO_4 Concentration (mg/l)	2.2	1.7 (1.5-1.9)	1.9 (1.9-2.0)	3.6	3.5 (3.2-3.6)	3.5 (3.2-3.5)	3.3 (2.8-3.5)	--
Percent H_3PO_4 of Aerosol Mass	76	70.5 (69-72)	74 (71-75)	72	74 (72-75)	71 (70-74)	71 (71-73)	--
Particle Size								
Mass Median Aerodynamic Diameters (μm)[b]	.8 (.8-.8)	.825 (.82-.83)	.79 (.68-.89)	--	--	--	--	ND
Respiratory Gases[c]								
O_2 (%)	19	19 (19-19)	19 (19-19)	19	19 (15-19)	19 (19-19)	19 (18-22)	19 (19-21)
CO_2 (ppm)	605	575 (545-605)	629 (569-641)	726	787 (726-847)	726 (605-847)	696 (605-847)	447 (387-484)
CO_2 - From-Burn (ppm)[d]	46	48 (0-48)	72 (12-85)	169	230 (169-290)	169 (48-290)	139 (48-290)	--

Table 7 (Continued).

	TARGET CONCENTRATION							
	3.0 mg/l 133 μm			6.0 mg/l 293 μm				0.0 mg/l
Extrusion setting Exposures	1	2	3	1	2	3	4	4
Contaminant Gases[c]								
CO (ppm)[e]	6	8 (5-12)	18 (6-18)	27	24 (ND-24)	27 (22-36)	24 (6-30)	ND
PH_3 (ppm)	ND	ND	ND	ND	ND	.1 (ND-.1)	ND	ND
C_6H_{14} (ppm)	61	18 (ND-18)	21 (ND-24)	73	45 (30-61)	61 (30-61)	30 (ND-61)	ND
Exposure Duration/ Chamber Conditions[f]								
Length of Exposure (min)	73	81	73	77	77	78	77.5	87
Temperature (C°)	(19-20)	(18-19)	(19-21)	(21-21)	(20-21)	(20-21)	(20-21)	(20-22)
Relative Humidity (%)	(50-52)	(52-58)	(49-60)	(60-60)	(48-56)	(50-58)	(52-64)	(48-52)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on these indices.

[b] Determinations of MMAD for each sampling and RP/BR burn were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained at the 293 μm extrusion rate due to the rapid overloading of particles on cascade impactor crystals obscuring the measurement. Small volume sample sizes were insufficient to determine MMAD values for filtered-air exposures (ND = Not Detected).

[c] All Gastec Analyzer Tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with manufacturer's recommendations. The formula for this correction was:

$$\text{Actual Tube Value} \times \frac{(760 \text{ mm Hg})}{(628 \text{ mm Hg})}.$$

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 Room 158 CO_2 readings; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting.

[e] The EPA standard for CO is 35 ppm maximum for a 1-h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were ≤ 30 ppm. This is somewhat unexpected for the 293 μm extrusion rates because Task 1 data revealed that a rate of 270 μm produced ≥ 30 ppm CO; this result is unexplained (ND = Not Detected).

[f] Median or range of values are presented.

605 ppm or between 696 and 787 ppm at the 133 and 293 μm extrusion pump settings, respectively.

Regarding contaminants, the rock dove RP/BR aerosol exposure data were almost identical in pattern to those described for prairie dogs. Phosphine was rarely detected, and only in trace amounts. The median CO values were very acceptable and varied between 6 and 27 ppm. Similar to the prairie dog exposure data, hexane was again present in RP/BR aerosols at unexpectedly high levels. Median C_6H_{14} measured values for the 133 and 293 μm extrusion settings ranged from 18 to 61 ppm and from 30 to 73 ppm, respectively. Again, we are unable to explain these levels of C_6H_{14} as measured by Gastec tubes. These values were disparate from both Sterner et al. (1988) data and the GC/MS data of Moneyhun et al. (1988).

Durations of the RP/BR-aerosol exposures for rock doves ranged between 73 and 81 min. Temperatures and RH values were well within acceptable limits during the exposures--18 to 22° C and 48 to 64 percent, respectively.

b. Mortality and Symptomatology

Mortality.--A total of 11 out of 42 rock doves died within 8 days following their last RP/BR-aerosol-exposure session. None of the 6 rock doves in the filtered-air-exposed (control) group died in the 30-day post-exposure period. Overall mortality results have been tabulated and are shown in Table 8. As indicated, 2 to 3 animals per group died apparently from the toxic effects of RP/BR-aerosol inhalation after whole body exposures for 1, 2, 3 or 4 daily 80-min sessions at the 6.0 mg/l target concentration. One animal died in the 3.0 mg/l group given 3 exposure sessions.

Our data strongly indicated that the male rock doves are more sensitive to the lethal effects of RP/BR aerosol than are the females. Comparing only the 7 RP/BR-aerosol-exposed groups, 10:24 males (41.7 percent) versus 1:18 females (5.6 percent) died within 5.4 ± 1.7 days post exposure. The difference in total numbers of rock doves of each sex exposed was the result of 3 males being mis-sexed by the cloacal examination method. These changes were incorporated into all rock dove analyses.

Symptomatology.--Because 11 rock doves died during the post-exposure periods, symptom data for different groups had to be compared based upon a computation of the percent of positive symptoms observed. These percentages were based on the total number of live birds in each group observed for each pre- and post-exposure 7-session block. The 7-day pre-exposure period (Pre-) was compared with the first 7 days of post-exposure (Post 1), and 7 late post-exposure sessions on Days 10, 13, 16, 19, 22, 25 and 28 (Post 2).

Table 8. Rock dove mortality data.[a]

Target RP/BR aerosol concentration	Number of daily exposure sessions	Male mortality ratio[c]	Post-exposure days until death	Female mortality ratio[c]	Post-exposure days until death	Total mortality ratio[c]
0.0	4	0:3	-	0:3	-	0:6
3.0	1	0:3	-	0:3	-	0:6
3.0	2	0:3	-	0:3	-	0:6
3.0[b]	3	1:4	5	0:2	-	1:6
6.0[b]	1	2:4	(7,8)	0:2	-	2:6
6.0	2	3:3	(5,6,6)	0:3	-	3:6
6.0	3	2:3	(5,6)	1:3	5	3:6
6.0[b]	4	2:4	(1,5)	0:2	-	2:6
Ratio totals		10:27		1:21		11:48

[a] The study actually involved 27 males and 21 females, rather than the intended 24 males and 24 females (i.e., both total nos. = 48 rock doves).

[b] One male in each of these groups was mis-sexed by the cloacal examination method at the start of the study. They were determined to be males at the end of the post-exposure period upon necropsy by APHIS veterinarians.

[c] Number of deaths : total number of birds tested.

Investigators were instructed to tape over data sheet headings for each RP/BR-aerosol or filtered-air group before recording observations in order to remove or reduce potential bias in reporting positive symptoms. However, another problem was noted in the data taken for the respiratory congestion symptom. This was scored in a high percentage of the observation periods (Post 1 and Post 2) for the filtered-air exposed doves (i.e., 44.8 and 54.5 percent respectively after flight exercise). While it is possible that a chronic respiratory problem could have existed for all birds in our colony, this explanation was considered unlikely when pre-exposure data were examined (i.e., 0.0 percent respiratory congestion symptoms). More likely, the investigators probably scored any evidence of breathing difficulty or even hyperthermia (e.g., parted beak, slight air rush sounds) as positive symptoms after the exposure period. The respiratory congestion measure was thus discounted as insensitive or as confounded in these symptomatology assessments.

The main symptomatology results are presented for the 7 RP/BR-aerosol and 1 filtered-air exposed groups in Table 9. Three main findings merit note based on these percentage data. First, all 7 RP/BR groups show some affected vocalization effects ranging from 2.2 to 12.5 percent. No filtered-air exposed doves showed this symptom pre- or post-exposure. These vocalization effects in the RP/BR groups dissipated by the Post 2 period. Second, 6 of the RP/BR-aerosol exposed groups showed 1 or more birds were affected in the body posture symptom category on Post 1. Thirdly, the 6.0 mg/l group, given 2 exposure sessions, appeared to be the most severely affected, and this was most consistently shown in the body posture symptom category. Percentages of observations that were positive for this symptom were 26.8 and 24.5 for the rest versus exercise observation counts, respectively, during the Post 1 period. The highest mortality rate (50 percent) was also noted for this group.

Other incidental changes that occurred included: an increased percentage of aggressive response in 6 of the groups (including filtered-air exposed doves) in the Post 2 period, and an increase in the percentage of dove normal vocalizations in 7 of the 8 groups during the Post 2 period.

Body weight effects.--Body weight data were analyzed for RP/BR aerosol exposure effects using 2 ANOVA designs. Both ANOVAs compared body weights on the last Pre-exposure Day (Day 0) with 14 Post-exposure sessions (Days 1-7, 10, 13, 16, 19, 22, 25 and 28). The first analysis involved 36 rock doves (6 groups) given 1, 2 or 3 exposure sessions to RP/BR-aerosol target concentrations of 3.0 or 6.0 mg/l. Only RP/BR-aerosol-exposed rock doves and no filtered-air-exposed (Control) rock doves were compared in this analysis. The design was a 2 (Concentration) x 2 (Sex) x 3 (Exposure) x 15 (Session) factorial, with Session treated as a repeated factor (Winer, 1971).

Table 9. Percentage[a] of positive symptoms in each category based upon the total number of observations in 7-session blocks for 8 rock dove groups before and after exposure. Individual groups received RP/BR target concentrations of 0.0, 3.0 or 6.0 mg/l for 1 to 4 successive daily exposure sessions.

Exposure Days	1			2			3			4		
Successive 7-Session Block	Pre-[b]	Post 1[c]	Post 2[d]	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2
	6.0 mg/l Target Concentration (293 µm Extrusion Setting)											
Body posture (rest)	0	0	0	4.5	26.8	4.7	0	3.5	0	0	3.5	0
Body posture (exercise)	0	0	2.8	0	24.5	9.4	0	5.8	0	0	2.8	0
Respiratory congestion (rest)	0	4.7	10.0	0	38.7	4.7	0	15.4	14.1	0	17.8	17.8
Respiratory congestion (exercise)	0	23.7	31.4	4.7	70.8	28.2	4.7	47.4	61.5	4.7	38.5	25.0
Plumage unkempt	2.2	4.7	3.5	0	0	0	0	0	0	0	0	3.5
Aggressive responses	4.5	0	17.1	4.5	10.2	23.5	23.4	7.1	23.5	0	0	0
Vocalizations	21.2	21.1	27.8	35.4	45.5	85.4	30.3	20.0	51.8	23.4	32.8	32.1
Normal	21.2	14.0	24.2	35.4	29.8	85.4	30.3	17.7	51.8	23.4	26.4	28.5
Affected	0	7.1	3.5	0	12.5	0	0	2.2	0	0	6.4	3.5
Lost	0	0	0	0	2.8	0	0	0	0	0	0	0
	3.0 mg/l Target Concentration (133 µm Extrusion Setting)									0.0 mg/l Target Concentration (Control)		
Body posture (rest)	0	2.2	0	2.2	4.5	2.2	0	2.2	0	0	0	0
Body posture (exercise)	0	0	0	0	2.2	2.2	0	2.2	0	0	0	0
Respiratory congestion (rest)	0	11.7	4.5	0	16.4	11.7	0	17.4	17.1	0	14.1	2.2
Respiratory congestion (exercise)	7.1	52.0	52.2	0	54.4	52.1	7.0	42.7	31.4	0	44.8	54.5
Plumage unkempt	0	0	0	0	0	0	0	2.2	0	0	0	0
Aggressive Responses	18.7	35.4	38.0	7.0	18.9	25.7	6.8	9.2	8.5	5.5	2.2	16.4
Vocalizations	40.1	61.5	52.0	37.7	66.2	63.8	21.1	25.0	8.5	28.4	40.1	47.4
Normal	40.1	52.1	49.7	37.7	54.4	61.5	21.1	19.7	8.5	28.4	40.1	47.4
Affected	0	9.4	2.2	0	11.5	2.2	0	5.1	0	0	0	0
Lost	0	0	0	0	0	0	0	0	0	0	0	0

[a] Because a few doves died within 1 to 8 days after RP/BR aerosol exposure, a percentage of the total number of observations made on the 6 rock doves on each 7-session block that were scored positive was used in place of frequency counts for each symptom category. This transformation has the effect of removing potential low count biases when doves died post exposure in certain groups.

[b] Refers to a 7-day period prior to RP/BR aerosol or filtered air exposure.

[c] Refers to the first 7-day period beginning on the day after the last RP/BR aerosol or filtered air exposure.

[d] *Refers to the Day 10 through Day 28 period with the day after the last RP/BR aerosol or filtered air exposure as the starting* date--(i.e., the 7 days include Days 10, 13, 16, 19, 22, 25 and 28).

Five significant effects were revealed by the analysis: Concentration x Sex x Exposure x Session ($F = 2.18$, $df = 20/271$, $P < 0.0029$), Concentration x Sex x Session ($F = 3.01$, $df = 14/271$, $P < 0.0003$), Concentration X Session ($F = 6.01$, $df = 14/271$, $P < 0.0001$), Exposure x Session ($F = 3.19$, $df = 28/271$, $P < 0.0001$) and Session ($F = 39.39$, $df = 14/271$, $P < 0.0001$).

Figure 12 depicts the components of the Concentration x Sex x Exposure x Session significant interaction term. All the other significant terms listed above are subsumed as components of this complex 4-way term.

As shown, the 4-way interaction and probably a large proportion of the 3-way interaction (i.e., Concentration x Sex x Session) stems from the fact that all of the males given 2 exposure sessions at the 6.0 mg/l RP/BR aerosol target concentration showed a dramatic and steady decline in mean body weight on Days 1 through 6 post exposure (see center panel, Fig. 12). By Day 7, all 3 males in this group were dead. All 3 females in this group, on the other hand, survived through the 28-day post exposure period and these female doves showed no reliable decline in mean body weight.

Another partial accounting of the 3- and 4-way interaction terms can be seen in the 3-Exposure Groups (see bottom panel, Fig. 12). In this case, 6.0 mg/l exposed males showed sustained depression of mean body weights on Days 10 through 28 when compared with the 3.0 mg/l exposed males. The corresponding female groups, however, showed no consistent differences in mean body weights on Days 10 through 28 post exposure, but the 6.0 mg/l group relative to the 3.0 mg/l group females did show more decline in body weight from their Day 0 mean value lasting through Day 7. Thus, the main 4-way interaction effect was generated by Sex interacting with the other 3 factors: Concentration, Exposure and Session.

The Exposure x Session interaction (Fig. 13) indicated that the groups given 1, 2 or 3 exposures were not equivalent in body weight means on the Pre-exposure Day (Day 0), confounding direct comparisons. Two effects, however, were apparent. First, both the 1- and 2-Exposure Groups showed equal declines from pre-exposure in mean body weight values through Day 5. The 2-Exposure Group was then consistently lower in mean body weight from Days 6 through 28. Both the 1- and 2-Exposure Groups never fully recovered from these body weight declines when compared with their respective pre-exposure day means. Second, the 3-Exposure Group, in contrast, showed relatively less mean body weight decline than the other groups, and these rock doves recovered from their initial mean body weight decline between Days 16 and 19 post-exposure.

The Concentration x Session interaction (Fig. 13) also indicated two general trends in the mean body weight values. The 6.0 mg/l

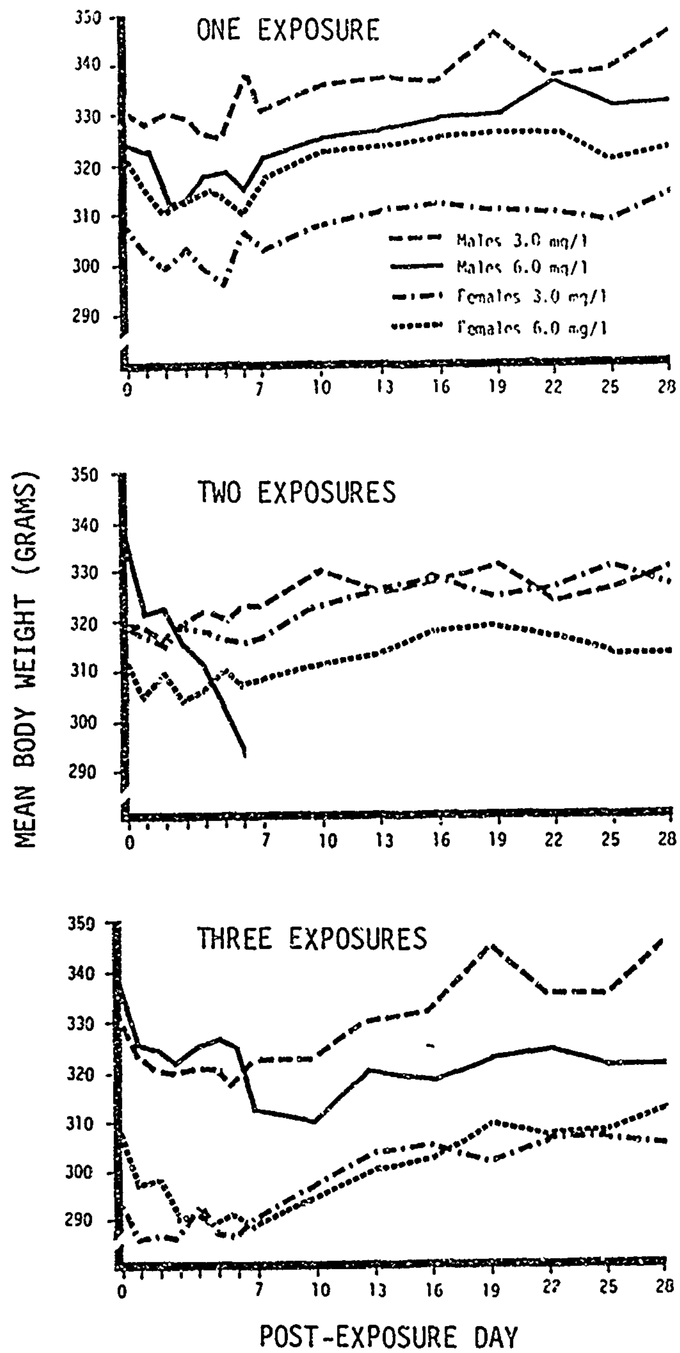

Figure 12. Mean body weights of rock dove groups given either 3.0 or 6.0 mg/l of RP/BR smoke concentrations on 1, 2, or 3 daily 80-min exposure sessions. Day 0 is the last pre-exposure day and Days 1-28 are the mean body weight values on the days following the last exposure day.

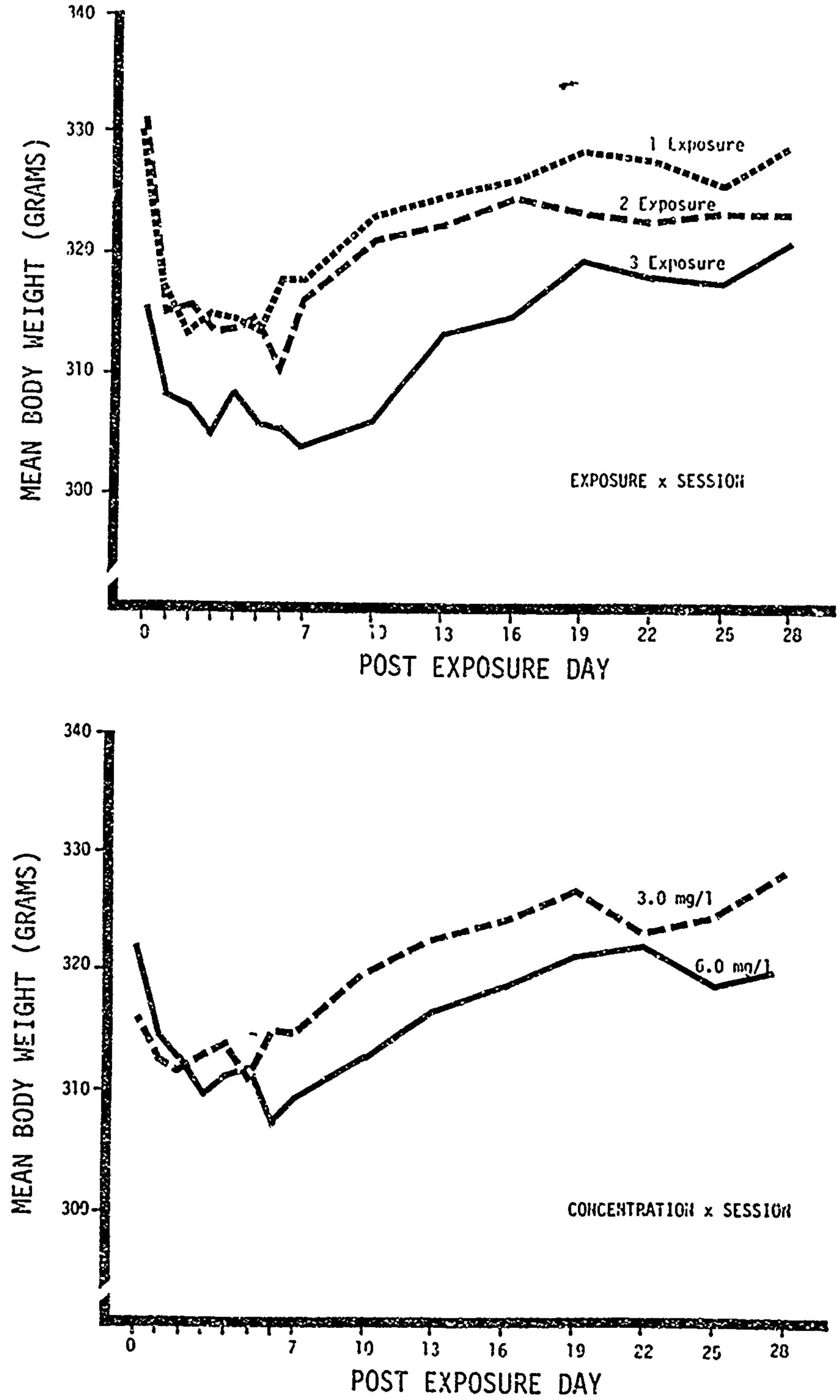

Figure 13. Upper panel.--Mean body weights of 3 rock dove groups that received either 1, 2 or 3 RP/BR-aerosol exposures (80 min each) at either the 3.0 or 6.0 mg/l target concentrations. Lower panel.--Mean body weights of 2 rock dove groups that received either 3.0 or 6.0 mg/l target concentrations of RP/BR-aerosol during 1, 2 or 3, 80-min exposures.

Exposure Group showed a greater decline in weight relative to pre-exposure, and the number of days to recovery to pre-exposure mean weights was different for the 2 groups. The 3.0 mg/l Exposure Group had recovered by Day 10; whereas, the 6.0 mg/l Exposure Group was not recovered until Day 22.

The Session effect indicated that the rock doves generally lost weight during the Post 1 period (Days 1-7) and recovered to pre-exposure weight levels during the Post 2 period (Days 10 through 28). Sustained Post 1 body weight depression was greatest in those groups that had high mortality--this factor limited our conclusions in tests for the Post 2 period exposure effects on the 6 rock dove groups.

A second repeated measures ANOVA involving 12 rock doves was performed on the body weight data for the 0.0 versus 6.0 mg/l target concentration groups which received 4 exposure sessions. The design was a 2 (Concentration) x 2 (Sex) x 15 (Session) factorial, with Session again treated as a repeated measures factor.

Four effects were found to be significant: Concentration x Sex x Session ($F = 1.84$, $df = 14/90$, $P < 0.0441$), Concentration x Session ($F = 2.38$, $df = 14/90$, $P < 0.0072$) Sex x Session ($F = 3.43$, $df = 14/90$, $P < 0.0002$) and Session ($F = 5.19$, $df = 14/90$, $P < 0.0001$).

The Concentration x Sex x Session interaction effect was further analyzed with Duncan's Multiple Range Tests (Waller and Duncan, 1969). Although the effect was complex with 60 means being compared to one another, definite trends were evident (see Fig. 14). As indicated, the 6.0 mg/l-exposed males showed significantly ($P < 0.05$) depressed body weights compared to the 0.0 mg/l group males on virtually all post-exposure days except Days 3 and 4. These 2 male groups were not significantly different from one another on the pre-exposure day. The 6.0 mg/l and 0.0 mg/l-exposed females, however, were neither significantly different from one another for the pre-exposure day nor for any of the post-exposure days, except for days 25 and 28 (i.e., the 6.0 mg/l, female group means were significantly lower on these last 2 days of post-exposure). This result indicated that males were much more consistently depressed in body weights than were females during the post-exposure periods. During these comparisons, 1 male died on the first day post-exposure and a second male died after 5 post-exposure days; no female RP/BR-aerosol-exposed birds or birds in the filtered-air (control) group died after the exposures.

The Concentration x Sex interaction indicated that for males, the 6.0 mg/l aerosol exposure produced an overall decline in body weight compared with the male 0.0 mg/l group birds. For females, however, the 6.0 mg/l aerosol essentially had no effect on body weight when compared to the female 0.0 mg/l birds.

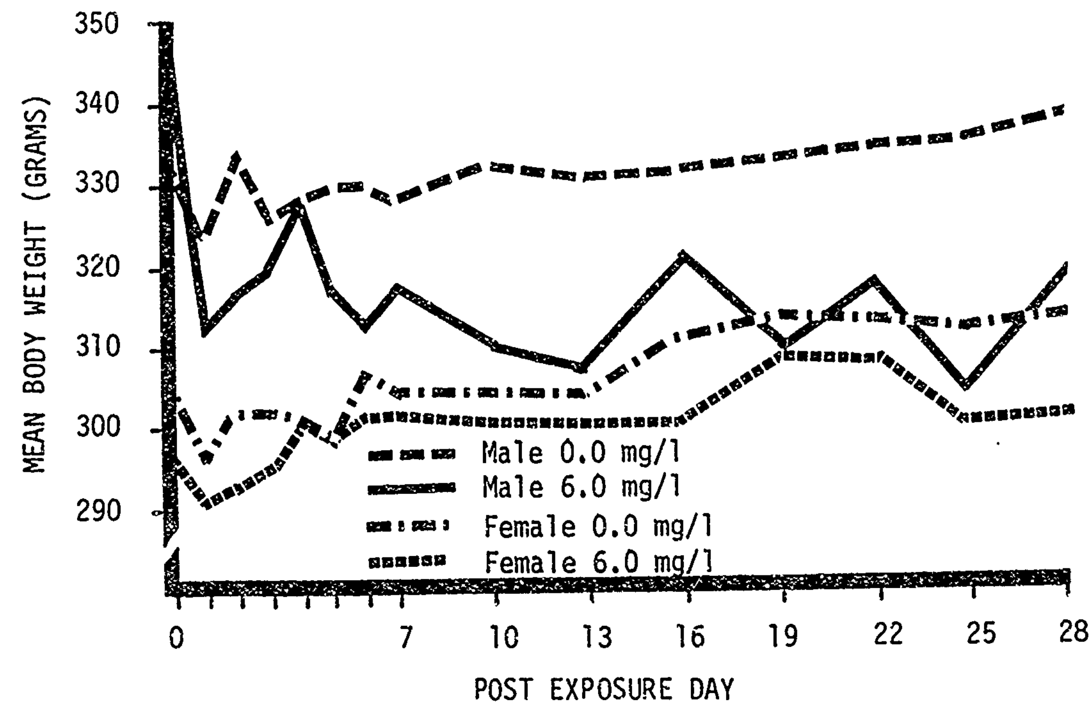

Figure 14. Mean body weights of rock dove groups all receiving 4 daily 80-min exposures to 0.0 or 6.0 mg/l of RP/BR aerosol as a function of the post-exposure time in days. The curves represent the Concentration x Sex x Session interaction effect. Whereas the female dove curves remain nonsignificantly different from one another (12:14 means), the male dove curves are significantly different ($P < .05$) on most of the post-exposure days (12:14 means).

The Sex x Session interaction again indicated that females showed a very slight gain in body weight over Sessions after a minor initial decline on Day 1. The males showed an initial pronounced decline in mean body weight on Day 1 followed by a variable but gradual decline on remaining post-exposure days. As a combined effect for all groups, the Session effect indicated that the mean rock dove body weights initially declined on Day 1 post exposure, recovered by Day 3 and were then stable through Day 28.

Water consumption effects.--The same initial 4-factor repeated measures ANOVA as described for the rock dove body weight data was also used to evaluate the water consumption data. Again, the design involved 36 rock doves (6 groups) given 1, 2 or 3 exposure sessions to RP/BR-aerosol target concentrations of 3.0 or 6.0 mg/l [i.e., a 2 (Concentration) x 2 (Sex) x 3 (Exposure) x 15 (Session) design] with no filtered-air exposed doves included.

Four significant effects were obtained from this analysis: Concentration x Exposure x Session ($F = 2.07$, $df = 28/271$, $P < 0.0017$), Concentration x Session ($F = 2.65$, $df = 14/271$, $P < 0.0012$), Exposure x Session ($F = 2.18$, $df = 28/271$, $P < 0.0008$ and Session ($F = 12.2$, $df = 14/271$, $P < 0.0001$). Note that all lower order effects are contained in the 3-way interaction term.

To analyze the Concentration x Exposure x Session interaction effect, 3 component 2-way interaction graphs were plotted as shown in Figure 15. These graphs represent the Concentration x Session effect, the Exposure x Session effect and the Concentration x Exposure effect.

As indicated, the Concentration x Session effect ($P < 0.0012$) shows that the 3.0 mg/l exposed rock doves were not affected to any degree during the first 7 days of post exposure. The 6.0 mg/l exposed rock doves showed a slight depression in water consumption through Day 6 post exposure. On Days 10 through 28, however, the 3.0 mg/l Group showed consistently elevated mean water consumption levels compared with the 6.0 mg/l Group.

The Exposure x Session effect ($P < 0.0008$) indicates 2 main trends in the data. First, the 1- and 2-Exposure Groups declined in water intake briefly for 1 to 2 days post exposure; whereas, the 3-Exposure Group showed no immediate post-exposure decline. Second, for almost the entire late post-exposure phase (i.e., from Days 7 through 25), mean water consumption levels are directly related to the number of RP/BR aerosol exposure sessions (i.e., more exposures led to higher, sustained water intake levels). This did not hold on Day 28, however, where the 1- and 2-Exposure Groups showed reversed consumption levels.

Finally, the Concentration x Exposure effect ($P < 0.63$; non-significant) indicated that, of all 6 rock dove groups tested, the 3.0 mg/l Group, given 3 exposures, drank considerably more

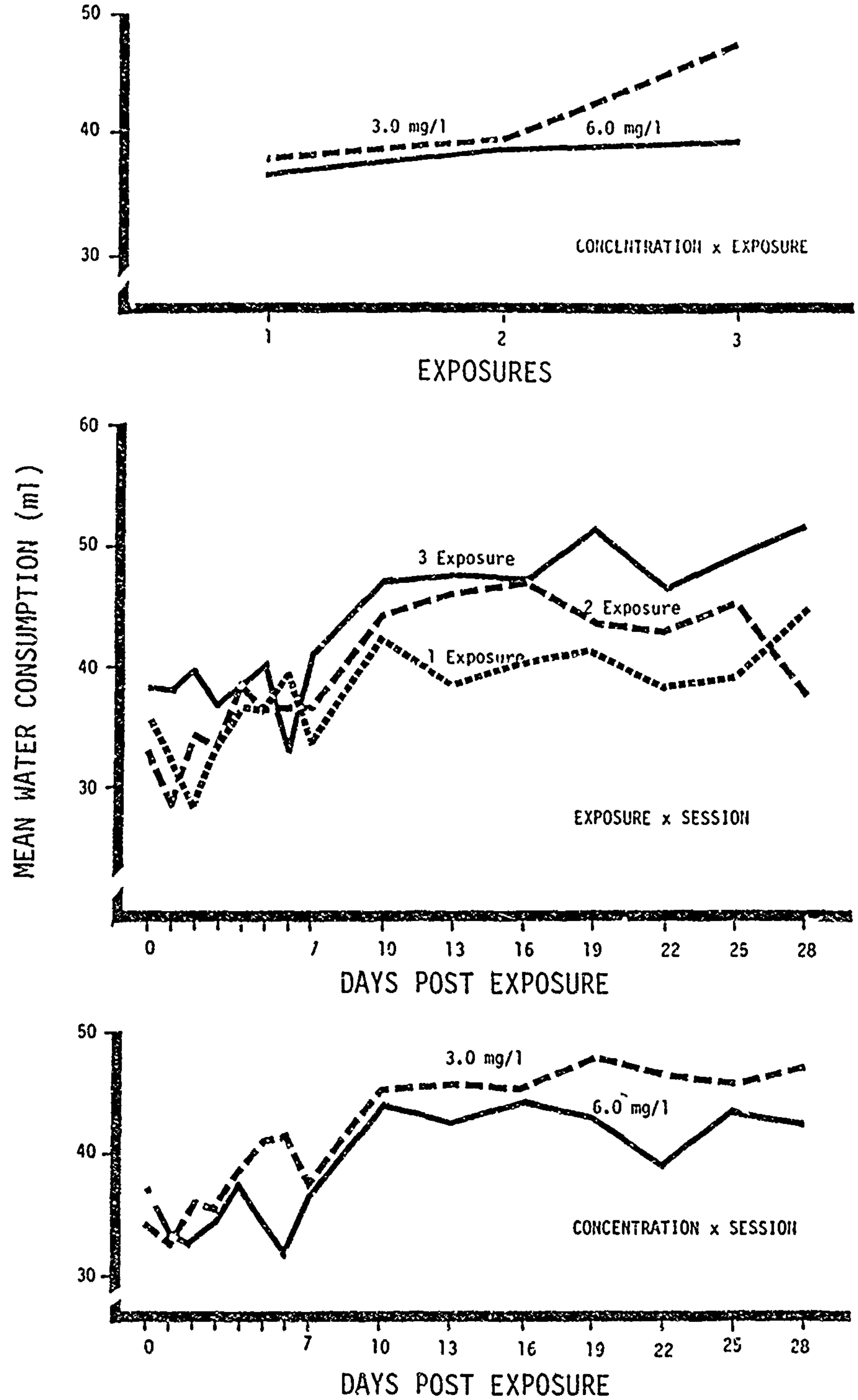

Figure 15. Upper panel.--Mean water consumption levels of rock dove groups exposed to 3.0 vs. 6.0 mg/l target concentrations of RP/BR aerosol for 1, 2 vs. 3, 80-min exposure sessions. Data for all sessions have been pooled. Middle panel.--Mean water consumption levels of rock dove groups that received, 1, 2 vs. 3, 80-min exposures. The 3.0 and 6.0 mg/l target concentration data have been pooled. Bottom panel.--Mean water consumption levels of rock dove groups that received 3.0 vs. 6.0 mg/l RP/BR aerosol. The 1, 2 and 3 exposure session data have been pooled.

water (mean increase value of 8.8 ml or 22.8 percent compared to other groups). Again, occasional extreme variability in water consumption levels for these 6 means over the 15 sessions negated significant differences.

The 3-way (Concentration x Exposure x Session) effect was therefore a function of the 2 significant, 2-way interaction effects: Concentration x Session and Exposure x Session. The major component of the complex interaction is attributed to late post-exposure effects shown by 3.0 mg/l and 3-Exposure Groups. The rock doves that received 3 exposures at the 3.0 mg/l RP/BR-aerosol level were, in fact, almost 23 percent above the mean consumption levels of the other 5 tested groups on Days 10 through 28 post exposure.

The explanation that appears most plausible for these elevated water consumption levels in the 3.0 mg/l Groups involves the high survivability of these rock doves post exposure (i.e., only 1 male died). These birds were affected late in the post-exposure period, and water consumption data were available for almost all of them. In the 6.0 mg/l Groups, in contrast, there was a relatively high degree of mortality (11:24 or 45.8 percent) affecting mainly males (64.2 percent of males versus 10.0 percent of females). These 6.0 mg/l Groups were thus culled of sensitive rock doves by Day 8 post exposure, and the remaining birds were probably selected for resistance to RP/BR-aerosol effects. This rationale would explain the lack of elevated water intakes in the 6.0 mg/l aerosol-exposed birds based on this selection process occurring during the post-exposure sessions. In other words, and as a cautionary note, it would be misleading to conclude from our data on rock doves that the general trend was toward higher water consumption levels at lower RP/BR-aerosol exposure levels when dealing with sublethal concentrations. For groups of doves that all survived the RP/BR exposures, in fact, the reverse may well be the case (i.e., higher RP/BR-aerosol concentrations could lead to more water consumption).

The Session effect reflected a very brief, slight depression (7.9 percent change) in mean water consumption on Day 1 followed by rapid recovery by Day 4. Rock doves then increased water consumption levels between Days 7 and 10 by approximately 20 percent and held steady at this increased level for the remainder of the post-exposure period.

A second ANOVA, essentially the same design previously described for rock dove body weight data, was also used to further analyze water consumption data. This 3-factor repeated measures design involved the remaining 2 groups that were given either filtered-air or RP/BR aerosol (6.0 mg/l target concentration) both on 4 exposure sessions. The design involved the factors of: 2 (Concentration) x 2 (Sex) x 15 (Session) with repeated measures on this last factor.

Only 1 significant effect was yielded by the ANOVA--Session ($F = 3.54$, $df = 14/90$, $P < 0.0001$). Waller and Duncan's Multiple Range test on the means showed that the birds drank significantly ($P < 0.05$) more water on days 10, 19, and 28 than on any of the other post-exposure days. This was an indication of a simple trend over time toward increasing mean water intake by both filtered-air and by RP/BR-aerosol-exposed rock dove groups.

c. Necropsies

As was the case with the prairie dog necropsies, a potential source of variation in the reported abnormalities for different groups should again be noted. These post mortem examinations were performed by a team of 3 veterinarians that were first guided through the procedures together, but later worked independently on different animal groups. The agreement among veterinarians in detecting and in describing organ abnormalities was not determined.

Post mortem examinations were performed on all 48 animals 30 days after the last RP/BR-aerosol or filtered-air exposures. APHIS veterinarians performing the necropsies were told which animals had received filtered air exposures, but were not informed as to the specific concentrations or number of sessions of RP/BR-aerosol exposure given to the other animals. As with the prairie dog necropsies, this information was requested by the veterinarians. It was viewed as the best course to take due to the need for their familiarization with necropsy identification of normal organs and tissues in rock doves.

The observed incidence and type of abnormalities in the 10 major organ groups examined are listed in Table 10. A few cases of tracheal excess mucus or exudate, along with 3 cases of enlarged spleens, were reported for the filtered-air exposed rock doves. This pathology was unexplained, but its occurrence made comparisons between RP/BR and control birds for these organs questionable.

The groups exposed to a 3.0 mg/l target concentration of RP/BR aerosol for 1, 2, or 3 daily sessions generally showed no increase in the incidence of pathology. However, 2 doves in the 1-Exposure, 3.0 mg/l Group had excess mucus in the larynx, and 1 bird had a large amount of white exudate material in the bronchi. No respiratory system pathology was reported for the 2-Exposure, 3.0 mg/l Group. Instances of liver abnormalities relating to color or texture were suspected to be artifacts of sodium pentobarbitol injections during euthanasia. One bird in the 3 mg/l, 3-Exposure Group had excessive exudate in both the larynx and in the epiglottis.

Table 10. Rock dove necropsy data. The incidence of each type of abnormality has been listed for the 8 groups (n = 6 animals/ea).

Target	Filtered-air exposed	RP/BR-aerosol Exposed						
Concentration (mg/l)	0.0	3.0	3.0	3.0	6.0	6.0	6.0	6.0
Number of exposures	4	1	2	3	1	2	3	4
Nasal passages							1M,1M+	1CE
Trachea	1M, 2FE	1M, 2FE			2M,1E+, 1CE+	1M+	1M,1CE, 1H	2M,1CE, 1FE
Larynx		2M		1E	3M,1CE,	1CE	1M, 1ED	1M, 1I
Epiglottis	1FE	2FE		1E	1CE+,1H		1CE,1DR, 1H	
Bronchi		1E+			1M	1CE+	1ED,1S+	
Lungs								
Heart						1CE		1DD
Liver		1GT	2DL			1DG	1GT,1DG	
Spleen	2S+, 1S+++	1L	1S+	1S+	1S+		1DD	1DG
Kidneys	1WN							

Key:

M = excess white/clear mucus
M+ = large amount of white mucus
E = thick creamy white exudate
E+ = large amount of white exudate
CE = catarrhyl exudate
CE+ = large amount of catarrhyl exudate
FE = fibrous exudate
ED = edemitis
GT = granular textured
I = inflammation
H = hemorrhages
L = lesions
S- = reduced size
S+ = enlarged/swollen
S++ = enlarged/moderately
S+++ = enlarged/highly
D = discolored
DL = light/pale/yellow color
DD = darkened color
DR = reddish color
DG = greenish color
A = small abscesses
WN = white ncdules/nodules

For rock doves exposed to a target concentration of RP/BR aerosol at the 6.0 mg/l level, there was a slightly increased frequency of mucus or exudate in the nasal passages and in the larynges in the 3- and 4-Exposure Groups. Some instances of increased reddish color or inflammation were also reported for the larynx, as well as one instance each of lesions and hemorrhage in this organ. Bronchi were reported to show one instance each of excess mucus, a large quantity of catarrhyl exudate, edemitis, and swelling. Liver pathology related to color and texture was reported, but again, this was probably induced by the i.p. injection just prior to necropsy.

d. Histopathology

Table 11 presents the frequencies of histological descriptors observed for lung, liver, trachea, larynx, nasal turbinates and epiglottis specimens of the 48 rock doves (i.e., 6 per group) assigned to 7 RP/BR-aerosol and 1 filtered-air-exposure conditions. Appendix D contains copies of the NVSL Reports from which this table was derived.

Unlike the descriptive percentages of histopathological descriptors offered for prairie dog tissues, the current account of rock dove histology is a clinical summary provided by NVSL pathologists (see Appendix D). Essentially, the pathologists concluded that none of the histopathology cited in Table 11 is attributable to RP/BR-aerosol exposures. Again, however, we must point out that the lack of standard (normal) histology slides for this wildlife species, coupled with the pathologists' unfamiliarity with insults due to RP/BR-aerosol inhalation, led to conservative interpretations of tissue specimens. The following is the verbatim account of the NVSL Laboratory Report concerning rock dove histopathology:

No significant lesions were found in nasal turbinates examined. Tissues from many pigeons were not available for microscopic examination.

No significant lesions were found in sections of epiglottis from control or exposed pigeons.

Lymphocytic inflammation was present in sections of larynx from one control and one exposed pigeon. This lesion is nonspecific.

No significant lesions were found in sections of trachea from control pigeons. Lesions found in sections of trachea from exposed pigeons included lymphocytic inflammation (2 pigeons), hemorrhage (1 pigeon), and fibrosis (1 pigeon). These lesions are considered to be nonspecific.

Hemorrhage was found in lung sections from four control and 15 exposed pigeons. This lesion may be related to the method of

Table 11. Frequencies of tissue classifications observed by NVSL pathologists for specimens obtained from rock doves administered single or multiple RP/BR and filtered-air exposures at the 133 and 293 µm extrusion settings for the toxicity range finding studies.[a]

RP/BR Target Concentration	3.0 mg/l			6.0 mg/l				0.0 mg/l (Filtered-air)
Extrusion Setting	133 µm			293 µm				
Exposures	1	2	3	1	2	3	4	4
Tissue								
Lung	1 NE	1 NL					2 NL	1 NE
							1 NE	
			1 A	1 A	1 A	2 A	1 A	
	4 H	2 H	3 H	1 H	3 H	1 H	1 H	4 H
	3 PC	1 PC	3 PC	2 PC	3 PC	1 PC		3 PC
		2 C	2 C	3 C	2 C	5 C	2 C	1 C
	1 C							
		1 PVLI		1 PVLI				
			1 E	1 E	2 E	4 E		
						1 T		
Liver					1 NL	1 NL		
				1 NE			1 NE	
			1 A	1 A	1 A	1 A	1 A	
	2 C		2 C	3 C	2 C	4 C	2 C	1 C
					1 E			
	5 PPI	5 PPI	5 PPI	1 PPI	3 PPI	1 PPI	2 PPI	6 PPI
	1 PPD	1 PPD	1 PPD			1 PPD	1 PPD	
	1 N	1 N	1 N			1 N	1 N	
	1 CS	1 CS		2 CS	1 CS			
Trachea	6 NL	6 NL	5 NL	4 NL	6 NL	5 NL	4 NL	6 NL
							1 NE	
			1 A	1 A		2 A		
							1 F	
			1 H	1 H				
			1 LI	1 LI				
Larynx	6 NL	6 NL	5 NL	5 NL	6 NL	5 NL	5 NL	4 NL
						1 NE	1 NE	1 NE
			[illegible]	1 A		1 A		
								1 LF
				1 I				

Table 11 (Continued).

RP/BR Target Concentration	3.0 mg/l			6.0 mg/l				0.0 mg/l (Filtered-air)
Extrusion Setting	133 μm			293 μm				
Exposures	1	2	3	1	2	3	4	4
Nasal	6 NL		3 NL	6 NL	3 NL	1 NL		
Turbinates		6 NE	3 NE		3 NE	5 NE	6 NE	6 NE
	6 NL	6 NL			2 NL	5 NL	4 NL	6 NL
Epiglottis			6 NE	6 NE	4 NE	1 NE	2 NE	

[a] All specimens were examined by APHIS Pathobiology Laboratory, NVSL, Ames, IA. Epiglottal tissues were not included in these examinations. Multiple histopathological descriptors were used with each specimen; hence, descriptor codes exceed numbers of animals for certain tissue by exposure conditions.

Key to histopathology:

- A - Post mortem autolysis
- C - Congestion
- CS - Cholestasis
- E - Edema
- F - Submucosal fibrosis
- H - Hemorrhage
- LI - Lymphocytic inflammation
- LF - Lymphofollicular proliferation
- N - Necrosis
- NE - Specimen not examined
- NL - No lesion seen
- PC - Pneumoconiosis
- PPD - Periportal inflammation
- PPI - Periportal hepatocyte degeneration
- PVLI - Perivascular lymphocyte infiltration
- T - Thrombosis

euthanasia. Pulmonary congestion and/or edema are presumed to be related to the method of euthanasia or hypostatic changes which occurred after euthanasia. Pneumoconiosis, a lesion found in three control and 12 exposed pigeons, is regarded as an incidental finding. Perivascular lymphocyte infiltrates were found in lung sections of two exposed pigeons. This lesion is considered to be nonspecific.

Lesions in the liver included periportal inflammation, a nonspecific lesion usually associated with a previous bacterial infection. Hepatic congestion is presumed to be related to method of euthanasia. Cholestasis was found in liver sections from five exposed pigeons. Cholestasis is a nonspecific finding. Periportal degeneration and necrosis was found in liver sections from five other exposed pigeons. This lesion was found in individuals from five different groups. The etiology of this lesion is unknown but does not appear to be related to level of exposure.

No lesions, attributable to red phosphorus/butyl-rubber smoke exposure, were found in the tissues examined.

D. Summary and Conclusions

1. Main Findings

RP/BR-aerosol and filtered-air measures during both prairie dog and rock dove exposure sessions indicated acceptable levels of concentration variation (< 20 percent) measured both gravimetrically and by titration for H_3PO_4. Other measures, such as particle size and respiratory gases present, indicated acceptable levels and close agreement with past studies using this same RP/BR extruder system (Sterner et al., 1988; Moneyhun et al., 1988). Contaminant gases measured during exposures were within acceptable limits except for C_6H_{14} as measured by industrial-type samping tubes ($\geq$100 ppm) at the 6.0 mg/l target concentration levels. Later measurements of this contaminant using GC/MS analysis (Moneyhun et al., 1988), however, indicated only low levels of C_6H_{14} ($\leq$5 ppm) at the high extrusion settings. The industrial tube measurements were suspected to have been influenced by other components in the complex RP/BR combustion product mixture.

Mortality rates for the two species studied were found to be vastly different for the RP/BR-aerosol target concentration range of 2.0 to 6.0 mg/l and for up to 4 repeated 80-min exposure sessions. None of the prairie dogs died at these levels over a 30-day post-exposure observation period. In contrast, 11 of 42 rock doves exposed to RP/BR-aerosol died within 5.4 $\pm$ 1.7 days after their last exposure. Only 1 of 18 died at the 3.0 mg/l RP/BR aerosol level, but 10 of 24 died at the 6.0 mg/l level. In addition there was a sex difference--RP/BR-aerosol exposures were lethal to 10 of 24 males (42 percent) but only to 1 of 28 females (6 percent).

Symptomatology results were partially similar for these 2 species. Vocalization effects in prairie dogs were most prevalent in the groups exposed to multiple 6.0 mg/l exposures. Vocalization effects in rock doves were exhibited to a minor degree in all RP/BR-aerosol exposed groups, and these effects dissipated after the first post-exposure week. Respiratory congestion was found to be a symptom associated with 6.0 mg/l RP/BR-aerosol exposure in the prairie dog groups. This symptom could not be consistently measured in the rock doves, but 6 of 7 dove groups did show body posture effects post exposure. This symptom was most evident in the rock dove group given 2 exposures at the 6.0 mg/l level. Mortality was also highest for this group (50 percent).

Body weight measures were much more affected in the rock doves vis-à-vis prairie dogs. Prairie dogs exposed to RP/BR-aerosol generally showed only a 1-day loss in weight gain followed by a rapid recovery to pre-exposure levels by the third day post-exposure. Also, the 1-Exposure Groups gained weight slightly faster than the 2-Exposure Groups during post exposure. Rock doves, on the other hand, showed a sex difference in their body weights after RP/BR-aerosol exposures. Males had much more depression of body weights, especially in the 6.0 mg/l Groups given 2 to 4 exposure sessions. In general, male rock doves at the 6.0 mg/l level showed high mortality, and survivors did not recover to their pre-exposure mean body weight levels for the duration of the 28-day post-exposure period. The 6.0 mg/l Groups, compared to the 3.0 mg/l Groups, were more severely depressed and did not return to their pre-exposure body weight levels until 22 days post exposure.

Water consumption measures for the 2 species yielded results in partial agreement. In prairie dogs, groups given multiple RP/BR-aerosol exposures consistently drank more post exposure than did the 1-Exposure Group, and this effect was most evident during Days 10 through 28. Similarly, rock dove groups given 1, 2 or 3 exposures were in an ascending order of water consumption on Days 7 through 25 post exposure.

Concentration X Session interaction effects on water consumption were significant for both species, also. However, these results were not consistent. The prairie dog groups showed highest consumption levels in the 6.0 mg/l Group late in post exposure (Days 10-28), and the 2.0 and 4.0 mg/l Groups showed depressed consumption levels (Days 22-28) compared with the Filtered-air Exposed Group. The rock dove groups, in contrast, showed higher consumption levels on Days 10-28 by the 3.0 mg/l Group compared to the 6.0 mg/l Group. These apparently opposite effects were assumed to have been partially generated by the high rock dove mortality at the 6.0 mg/l level. Those doves that may have shown even more elevated drinking late in post exposure had died within the first week post exposure. Prairie dog data were not limited by this mortality factor.

Necropsy data analyses yielded no strong, consistent effects in either species. The only notable increase in frequency of abnormalities in the 10 organs examined was excessive mucous or exudate in the nasal passages and in the larynges of those rock doves exposed to the 6.0 mg/l level for 3 and 4 sessions. Histopathology data analyses indicated no specific pathology that related to RP/BR-aerosol exposures in 7 types of organ tissue examined for each species.

2. Inter-species Comparisons

Mortality data indicated that both species have higher LC_{50} values for RP/BR-aerosol than do Sprague-Dawley albino rats. Burton et al. (1982) and Aranyi et al. (1983b) reported LC_{50} concentration values of 2.46 mg/l and 2.32 mg/l for laboratory rats given 4 or 5 daily 1-h exposure sessions, respectively. Our data indicated that a median value of 4.6 mg/l RP/BR-aerosol concentration measured gravimetrically over 77 to 86 minutes (target concentration of 6.0 mg/l) did not kill any prairie dogs (n = 6) after 4 exposure sessions. The rock dove data indicated that a median value of 4.6 mg/l RP/BR aerosol concentration measured gravimetrically over 77 to 78 minutes (target concentration of 6.0 mg/l), killed 2:4 males and 0:2 females after 4 exposure sessions. The listed 80-min concentration values (Tables 3 and 7) in the current study are considered to be rather conservative estimates in terms of the steady state levels achieved in the exposure sessions. For all male rock doves exposed to the 4.5 to 5.0 mg/l RP/BR measured aerosol concentrations over 1, 2, 3, or 4 exposure sessions, 10:24 (i.e., 42 percent) died, indicating that these concentration and exposure ranges were lower than the estimated LC_{50} value for this species.

Aranyi et al. (1983b) reported that no observable clinical symptoms (other than occasional eye-crusting) were shown by albino rats after exposures to RP/BR aerosols at near lethal levels. As previously indicated, respiratory congestion and affected or lost vocalization symptoms were detectable in prairie dogs after exposure to the 6.0 mg/l target concentration levels. Rock doves exhibited vocalization effects and body posture symptoms post exposure.

Aranyi et al. (1983b) also reported mean body weight losses in male rat groups after exposure to between 1.56 and 3.05 mg/l of RP/BR-aerosol levels over 5 daily, 1-h exposure sessions. Body weights of female rats were less affected by these RP/BR-aerosol levels. As indicated, prairie dogs exposed to target concentrations up to 6.0 mg/l did not show strong, reliable body weight losses post exposure. Rock dove body weights, on the other hand, were affected by RP/BR-aerosol exposures with the males showing much greater mean losses than females at the 6.0 mg/l target concentration level.

Burton et al. (1982) reported epiglottal and laryngeal ulcers, as well as pulmonary congestion, edema, and hemorrhages in albino rats exposed to 3.1 to 8.5 mg/l doses of RP/BR smoke. Our gross necropsy results indicated a minor degree of lung congestion in some prairie dogs and excess mucus or exudate in the nasal turbinates and larynges of rock doves 30 days after exposure to 4.2 to 5.0 mg/l median RP/BR smoke concentrations. Aranyi et al. (1983b) reported no incidences of gross pathology in rats exposed to 0.5 mg/l RP/BR smoke for up to 3.5 h.

Our histopathology results also indicated no increased incidence of pathology in RP/BR-aerosol exposed prairie dog groups. The Burton et al. (1982) study on albino rats, in contrast, reported an increased lung hemorrhaging incidence. Histopathological data reported by Aranyi et al. (1983b) for lung tissue were equivocal due to a suspected infectious agent present in their rat colony.

3. Selection of Task 3 Exposure Conditions

The level of RP/BR aerosol target concentration recommended as a maximum for Task 3 RP/BR studies was determined to be 4.0 mg/l. This level was mainly derived from an examination of the carbon monoxide (CO) concentration levels measured during both Task 1 and 2 studies. Levels of CO approached or exceeded 35 ppm on several RP/BR burns, when the extruder was set to deliver the 6.0 mg/l target concentration. This level of CO was deemed to be too high for a relatively pure RP/BR-aerosol effects evaluation since this approaches or exceeds the EPA safety standard for humans of 35 ppm for a 1-h average exposure (NRC, 1977).

In addition to CO level as a rationale for selecting 4.0 mg/l as a maximum target concentration, the 6.0 mg/l level would produce 33 to 50 percent mortality in rock dove groups--an unacceptable level for Task 3 studies on behavioral and physiological effects. The 4.0 mg/l level is expected to cause minimal mortality in rock doves when given for only 2 daily, 80-min exposure sessions; this level is also expected to cause no mortality in prairie dog groups exposed over 4 daily, 80-min sessions based on the current Task 2 results.

As a minimum target concentration for Task 3 studies, the 1.0 mg/l RP/BR-aerosol level is recommended. This level is desirable from 2 standpoints. First, it allows for an examination of the sublethal effects of RP/BR-aerosol exposure at a relatively low level using potentially more sensitive measures (e.g., pulmonary function and startle response) than those used in Task 2; and second, 1.0 mg/l was found to be close to the minimum concentration level that will allow steady burning of the extruded RP/BR ribbon in the burn chamber. Thus, flame out and re-lighting problems would largely be eliminated at this concentration level, so that a high degree of temporal homogeneity of RP/BR-aerosol can be maintained during the Task 3 exposures.

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IV. APPENDICES

Appendix A. Excerpts from DWRC Work Unit 615.16 (i.e., Methods)--Effective Smoke Concentration Range Finding and Basal Physiological Determinations (Behavioral and Physiological Effects of Red Phosphorous Butyl-Rubber (RP/BR) Smoke on Two Wildlife Species--Task 2)--plus a copy of the Report of Animal Care Committee approving the Task 2 research studies.

A. Animals and Animal Care Procedures

Numbers.--Approximately 110-120 prairie dogs and 110-120 rock doves will be used in Task 2. The sub-lethal RP/BR exposure phase will involve 48 animals of each species and the basal physiological determinations will involve 70-80 animals of each species (Fig. 1).

Capture.--Black-tailed prairie dogs and rock doves will be caught locally by Project Staff. Every effort will be made to insure the humane care and treatment of animals throughout all phases of the research. All cages and handling procedures will concur with the current regulations concerning the Animal Welfare Act. The attending veterinarians are: Drs. Gary W. Church and Patricia L. White, Veterinary Service, Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), Rm. 237, 2490 W. 26th Avenue, Denver, CO 80221. Prairie dogs will be captured at several known colonies in the Denver Area; landowner permission will be obtained prior to these efforts. In general, prairie dogs will be caught using 1 of 2 methods: (1) foaming of burrows or (2) trapping.

The foaming method will involve dispensing a narrow stream of laundry detergent with water into burrow openings known to be occupied by prairie dogs. The suds cause prairie dogs to leave the burrow and the animals are then hand captured with snares and gloves. The repellent action of the detergent has been judged to be non-traumatic and the concentration contacting the animals is less than 0.5 percent as it is mixed with water.

The trapping method will involve positioning #203 Tomahawk Traps (61 x 15 x 15 cm) at burrows during daytime. All traps will be covered with white plastic tops to provide shade for animals during trapping in hot weather. Traps will be baited with food, then checked daily at dusk for the presence/absence of a prairie dog. In hot summer weather or cold winter weather, traps will be checked twice daily (i.e., approximately 1300 and 2000 h and 1300 and 1700 h, respectively) to reduce effects of heat and cold stress in captured prairie dogs. The diurnal activity of the animal precludes night trapping. Following capture, animals will be dusted for ectoparasites with Purina Dog Powder (a.i. pyrethrins 0.1%, piperonyl butoxide 1.0% and carbaryl 5.0%) and then transferred to individual holding cages (91 x 61 x 20 cm) for transport to DWRC.

Rock doves will be purchased from a local supplier and housed in 3.0 x 1.5 x 1.8 m 5.1 cm wire mesh cages in a covered outdoor facility. Density of pigeons in each cage will not exceed 30 birds. They will be maintained on Purina pigeon checkers and cracked corn with water and grit available _ad libitum_. Each wire holding cage shall contain sufficient perch space for 30 birds and an attached 1.0 x .75 x .75 m wood shelter box. After at least 2 weeks of holding in these outdoor cages the birds will be moved to the inside quarantine aviary cage facility.

Quarantine and Holding of Animals Prior to Test.--Upon arrival at DWRC, prairie dogs will be permanently segregated by sex. Before the quarantine is initiated, these animals will receive a second dusting with the Purina Dog Powder. Each animal will be weighed (nearest g), implanted with a transponder by injection for identification, and checked for outward signs of disease and poor health before the Quarantine Period is started. Unhealthy animals will be euthanized using ether and then incinerated. Throughout the period of research, the prairie dogs will be maintained on water and Purina rabbit checkers (Performance Blend; crude protein of not less than 17.0%; crude fat not less than 2.0%; crude fiber not more than 18.0%; and ash not more than 8.0%).

Quarantine procedures will adhere to the following 10 recommended practices of the DWRC Animal Care Committee:

1. The quarantine area will be isolated from the main research area (Bldg. 16) to provide adequate biosecurity. Animals will be checked daily, and any problems (e.g., health, security) will be reported to the Principal Investigator. Quarantine will begin and end with no animals admitted or removed from the original group in the quarantined area until completion of the Period.

2. The Quarantine Period will be 14 days in length. This will allow for the detection of parasitic and disease problems, as well as, time for the animals to adjust to their captive diet and surroundings.

3. All incoming animals will be examined by one of the Consulting Veterinarians for signs of disease and injury prior to Quarantine. Initial external parasite control will be started either in the field before transport or upon arrival at DWRC depending upon air temperature and length of time elapsed from exposure to the detergent foam treatment. All animals will be re-dusted prior to onset of the Quarantine period.

4. Tests will be performed on pooled fecal samples of all prairie dogs to be quarantined in order to detect potential internal parasites; if positive, the entire group of animals will be treated with an appropriate antiparasitic drug.

Pooled rock dove fecal samples will be submitted to the National Veterinary Services Laboratory to be inspected for Velogenic Viserotropic Newcastle Disease (VVND) and Psittacosis. If positive for VVND the entire group of birds will be euthanized using ether and incinerated; all cages, bedding, food, and any other materials in contact with the birds will be disinfected or incinerated. If positive for Psittacosis, each bird will be assessed individually with cloacal swabs; those birds showing positive confirmation will be euthanized.

5. All unusual symptoms and deaths of quarantined animals will be promptly reported and recorded by the Principal Investigator. Post mortems and diagnostic workups will be performed, if indicated by the consulting veterinarians.

6. Food storage will be located adjacent to the Quarantine Area and not in contact with food to be given to nonquarantine animals. Storage of other supplies and water containers used will also be located in the Quarantine Area.

7. Vermin control will be strictly monitored and enforced using glue boards and traps in the Quarantine Areas. Insect control will be accomplished using appropriate insecticides prior to the Quarantine Period.

8. Respirator masks must be worn by personnel at all times when working around avian subjects in the Quarantine Area.

9. All personnel will change their outer wear clothing including boots, before entering and leaving the Quarantine Area.

10. After the Quarantine Period is over, the entire Area will be cleaned and disinfected. This includes cages, holding pens, water and food dishes.

11. Animals showing signs of debilitation or poor health following these quarantine procedures will be euthanized, necropsied, and incinerated. All animals displaying good health will then be available for transport to appropriate laboratory animal colony holding rooms in Building 16.

Prairie dogs will be held in a separate heated and air-conditioned brick building on the DFC. This building is temperature ($23^{o} \pm 5^{o}$ C) and light controlled. The animals will be reweighed every 14 days; animals losing more than 15 percent body weight will not be used for research until determined "healthy" by the consulting veterinarian. Lactating or pregnant females will not be used in research studies. These animals will be euthanized using ether.*

Regarding rock doves, each will be checked for outward signs of disease and poor health, dusted with Purina Dog Powder, weighed, and banded for individual identification before the Quarantine Period is initiated. Throughout the period of research, doves will be maintained on water and Purina Pigeon Checkers with grit available *ad libitum*.

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* Sodium pentobarbital injection substituted for ether during actual work.

Quarantine of rock doves will involve placing the males and females in either of three wire mesh aviary cages (1.6 x 3.3 x 2.6 m; 2.0 x 6.6 x 2.6 m; 3.9 x 3.9 x 2.6 m) located within a 11.5-m-diameter Butler building on the DFC. At the end of the Quarantine Period and each 14-day interval of captivity, all birds will be re-weighed. Those animals losing more than 20 percent body weight (i.e, relative to initial weight) will be held until weight and status are acceptable, then made available for research studies. All birds showing severe body weight loss or other unhealthy signs will be treated as appropriate to restore health or euthanized. Animals not responding to veterinary treatment will be euthanized and incinerated. All doves not surviving Quarantine will be refrigerated for later necropsy examination by the Veterinarians. Birds appearing healthy and maintaining body weight after the 14-day Quarantine Period will be available for RP/BR smoke exposure tests in Building 16.

Holding of Animals During Test.--During specific studies, animals will be separately housed in rabbit-type cages (Hazleton Systems H-1432, Aberdeen, MD--61 x 62.5 x 41 cm; Wahman Mfg. Co., Baltimore MD--51 x 54 x 38 cm) and allowed 14 days adaptation prior to use in experiments. The research will be conducted in isolated rooms of Building 16. Throughout the studies, all prairie dogs and doves will be maintained on a 12:12-h light/dark schedule, with temperature controlled at $23^{o} \pm 2^{o}$ C. A restricted feeding schedule will be maintained with food available between the hours of 0630 and 1430 MST (8 hrs) to counteract over eating by these individually caged animals.

Use of Anesthetics and Euthanasia Drugs.--If possible, pain alleviating drugs will not be used during behavioral or physiological measurement procedures because of potential distortion of measured effects. If and when drugs are administered to animals, specific drug and dose procedures will be provided as Standard Operating Procedures to the contractor, and appropriate control groups will be included in these designs. In the case of severe debilitation in the animals produced by RP/BR smoke exposures, the animals will be observed daily for recovery or mortality. Euthanasia would not be used as this would obviate mortality-effects data

For collection of blood specimens during certain physiological procedures, animals will be restrained. If restraint proves overly stressful to the animals, Methoxyflurane will be used to anesthetize both species.*

For necropsy examinations the drug to be used for euthanasia of prairie dogs will be sodium pentobarbital administered according to the procedures recommended by the American Veterinary Medical Association (Smith, Houpt, Kitchell, Kohn, McDonald, Passaglia, Thurmon, Ames, 1986). Cervical dislocation will be used as the means of euthanasia with pigeons for necropsy purposes.**

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* Report of blood analyses to be included in Task 3 Report.

** Cervical dislocation was replaced with sodium pentobarbitol injection for euthanasia of rock doves.

REPORT OF ANIMAL CARE COMMITTEE

United States Department of Agriculture

Animal and Plant Health Inspection Service

Animal Damage Control

Denver Wildlife Research Center
Bldg. 16, Denver Federal Center
P.O. Box 25266
Denver, CO 80225-0266

Principal Investigator:	Research Study Title:
S. A. Shumake, R. T. Sterner B. E. Johns	Effective Smoke Concentration Range Finding and Basal Physiological Determination. Task 2

This committee has reviewed the above described project with respect to the rights and safety of the animal subjects. The following are our findings:

Risks (Check one)

___ The planned research involves little foreseeable risk and adequate precautions have been taken for the safety and comfort of the subjects unless the plan is modified.

X The foreseeable risk is justified by the anticipated benefit to society and the plans include adequate and appropriate measures to ensure the comfort and safety of the subjects insofar as feasible.

___ The risk is justified but further measures seem advisable to protect the subjects. Comments are attached.

___ The risk seems greater than can be justified by the research as planned and the research study is not approved as presented.

Further Comments:

Recommendation (Check one)

X The research study be approved as submitted.

___ The research study be revised in keeping with our comments and resubmitted.

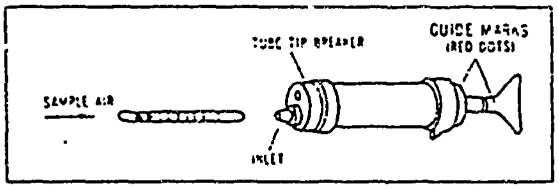
___ The research study as described be rejected.

Signature	Title	Date Signed
Signature of Committee Chairperson Since member of research team, Brad E. Johns did not sign. BEJ	Research Physiologist	4-17-87
Signature of Committee Veterinarian Gary W. Church	Veterinary Medical Officer, VS, APHIS, USDA	5/6/87
Signature of Committee Member Peter J. Savarie Peter J. Savarie	Pharmacologist	4/22/87
Signature of Committee Member James E. Davis	Biological Laboratory Technician (Wildlife)	4-22-87
Signature of Committee Member Jennifer Keller Jennifer L. Keller	Biological Aid (Animal)	4-22-87
Signature of Committee Member Louis Ray Burke	Representative of Community	4/20/87

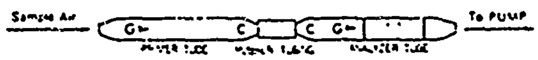
Appendix B. Operating Guide for the Gastec Analyzer Pump and Specifications for O_2, CO_2, CO, PH_3 and C_6H_{14} Analyzer Tubes. (We thank Sensidyne, Inc., the U.S. distributor for Gastec Corp., for permission to print these instructions/specifications.)

OPERATING INSTRUCTIONS
GASTEC PRECISION GAS DETECTOR SYSTEM

SAMPLING & MEASUREMENT PROCEDURE:



1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert tube securely into pump inlet with arrow on tube pointing toward pump.
3. For twin tubes, connect Ⓒ marked ends with rubber tubing after breaking each end. Insert analyzer tube into pump with arrows on tubes pointing toward pump. See figure below.



4. Make certain pump handle is all the way in. Align red dots on pump body and handle.
5. Pull handle out to desired stroke. Handle can be locked on either 1/2 pump stroke (50 cc) or 1 pump stroke (100 cc).
6. Read concentration at the interface of stained-to-unstained reagent when staining stops. Unlock handle by making 1/4 turn and return it to starting position.
7. In case more pump strokes are indicated in instruction sheet in each box of tubes, take additional sample by repeating pump strokes without removing tube.

CORRECTION FOR TEMPERATURE, HUMIDITY OR PRESSURE:

Calibration of the Gastec detector tubes is normally based on a tube temperature of 20°C (68°F), approximately 50% relative humidity, and normal atomospheric pressure.

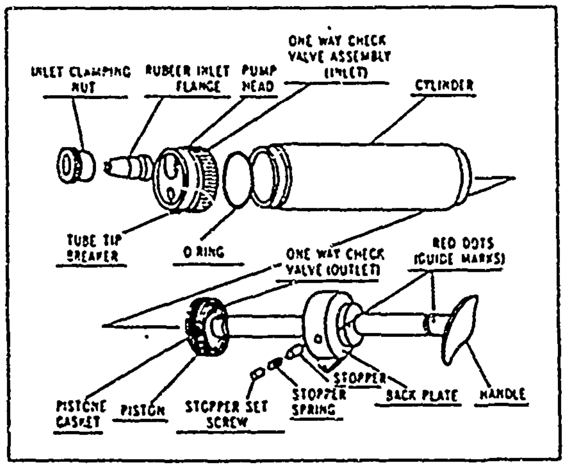
1. No correction is normally required for tube temperatures of 0°–40°C (32°–104°F) and for relative humidity range of 20–90%.
2. Where detecting reagent is abnormally sensitive to temperature or humidity, correction table or chart is provided in each box of tubes. In this case, tube reading must be corrected using correction table or chart.
3. Tube reading is proportional to absolute pressure. To correct for pressure, multiply by

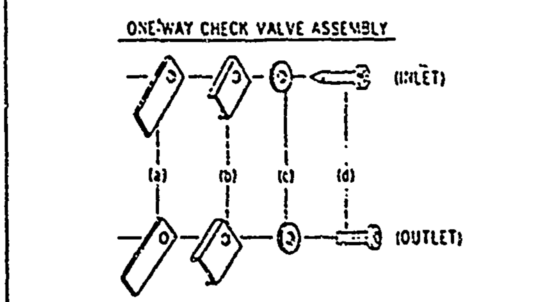
$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}$$

GASTEC PUMP PERFORMANCE

DESCRIPTION OF PUMP

Construction of pump is illustrated below. Pump pulls the highest vacuum (8.1" of Hg). It eliminates flow-rate orifice which may cause malfunction of pump by clogging or leaking orifice. Friction-proof piston gasket (lubricant seal packing) provides completely leakproof sampling at all times.





(a) Rubber valve flap
(b) Valve retainer
(c) Plain washer
(d) Valve screw

CHECKING PUMP PERFORMANCE

A. Visually check rubber inlet flange for cracks or tears. Replace if damaged. Tighten inlet clamping nut.

B. Valve Leak Check

1. Insert a fresh sealed detector tube into pump. Misalign red dots on pump and handle. Pull several fairly rapid continuous full pump strokes.
2. Pull handle out 6 mm (1/4 inch) and hold in this position for 1 or 2 seconds.
3. Release handle.
4. If handle returns to within 1.5 mm (1/16 inch) of fully closed position, continue to step C.

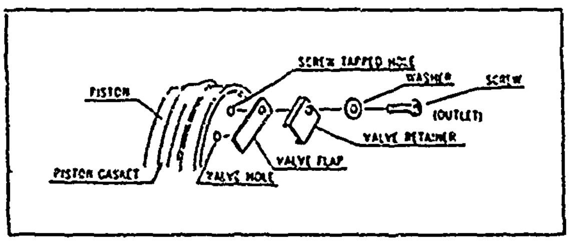
5. If handle does not return to within 1.5 mm (1/16 inch) of fully closed position (or less), perform the following Valve Lubrication instruction outlined below.

C. Field Volume Check

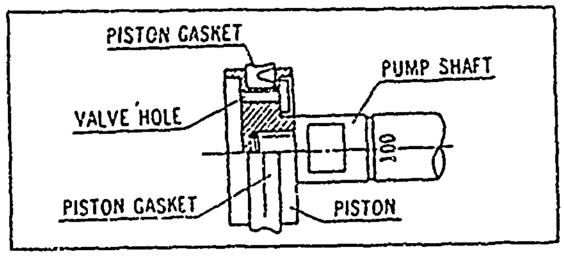
1. Insert a fresh sealed detector tube into pump.
2. Align red dots on pump body and handle.
3. Pull handle firmly and at a moderate speed until handle locks into position. Wait 1 minute.
4. Unlock handle by turning it and guide it back.
   TO PROTECT PUMP STOPPER from breakage, do not release the handle and allow it to spring back when conducting a leak test. Make sure to hold your hand onto the handle and guide it back.
5. Pump handle should return to within 6 mm (1/4 inch) of the fully closed position.
6. If pump handle does not close to within 6 mm (1/4 inch) or less, follow lubrication instructions and retest.

D. Lubrication Instructions (Perform Laboratory Volume Check "E" after each lubrication)

1. Valve Lubrication
   a. Unscrew back plate and withdraw piston from pump cylinder.
   b. Remove check valve from piston.
   c. Clean valve and piston with lint-free cloth. Proper valve cleansing is as follows: Place cloth flat on desk. Wipe rubber valve flap in a flat position across cloth. Do not bend the rubber flap valve.
   d. Apply a small amount of grease evenly around the valve opening to form a thin film. A thin film is nearly invisable.
   e. Replace valve assembly loosely in the same manner as removed.



   f. Before tightening the screw, align valve so that valve hole is in center of valve flap.
   g. Then push the rectangular valve retainer all the way toward loose end of valve flap.
   h. Now tighten screw. If a torque driver is available, tighten to 0.8 Kg-cm. Otherwise, be careful not to overtighten screw. When tightened, screw must not deform rectangular valve retainer.
2. Piston Gasket Lubrication
   a. Wipe off piston and cylinder with a clean lint-free cloth.
   b. Remove piston gasket with a small bladed screwdriver. Take care not to cut gasket.
   c. Clean slot in piston with lint-free cloth. Wipe off rubber gasket.
   d. Wipe an ample supply of grease into gasket slot on piston and inside gasket.
   e. Replace gasket making sure that open side of gasket is toward pump handle.



   f. With the excess grease from piston slot, wipe around outside of gasket and piston.
   g. Wipe an ample amount of grease into cylinder at the area of piston entrance.
   h. Insert piston slowly into the cylinder. Work the piston back and forth slowly in the cylinder several times.
   i. Now screw back plate firmly onto cylinder.
   j. Repeat leak tests.
   k. If any leak remains, replace piston gasket.
   l. Only if a leak persists, go to procedure below.
3. Pump Head Lubrication
   a. This is only necessary where all previous procedures have failed to correct a leak.
   b. Visually check pump head "O" ring for cracks.
   c. Replace "O" ring if cracked.
   d. Place a light coat of grease on pump cylinder head screw threads and the "O" ring.
   e. Insert new "O" ring.
   f. Screw pump head firmly on to "O" ring and make sure "O" ring is seated uniformly. Overtightening pump head may push "O" ring out of place. Do not overtighten.
   g. Wipe off excess grease.

E. Laboratory Volume Check (To be performed at least after each lubrication)

The Gastec pump can be checked periodically to assure that 100 ± 5 ml are being sampled.

1. Arrange a graduated 100 ml soap film flow meter in a volume test mode.
2. Insert a fresh Gastec tube into the Gastec pump. The tube must be broken at both ends (ready for use).
3. Attach the Gastec tube to top of soap film flow meter with rubber hose. Make sure there are no leaks.
4. Pull pump handle out full to lock at one stroke in normal sampling manner.
5. Wait until the bubble stops moving and read the volume evacuated.
6. If the volume evacuated is other than 100 ± 5 ml, proceed to lubrication instruction and retest.

GASTEC

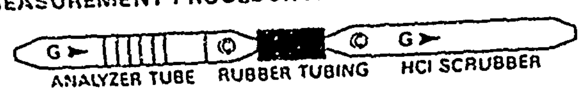
OXYGEN DETECTOR TUBE NO. 31

The Gastec Detector Tube No 31 provides a rapid fully quantitative analysis of the concentration of OXYGEN in air with an accuracy tolerance of ± 2% utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	6–24% (based on 1/2 pump stroke)
Measuring Range	6–24%
Number of Pump Stroke	1/2 pump stroke only (50 ml)
Detecting Limit	6% at half pump stroke (50 ml)
Sampling Time	1 minute per pump stroke
Color Change	Black—White
Shelf Life	2 years

MEASUREMENT PROCEDURE:



1. Break tips off a fresh analyzer tube and a HCl scrubber tube by bending each tube end in the tube tip breaker of the pump.
2. Connect the ends of the analyzer tube and HCl scrubber tube, marked with ©, using a rubber tubing supplied. Insert the HCl scrubber tube securely into the rubber inlet of the pump with the arrows on the twin tubes pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and pump body.
4. Pull the handle until it locks at half pump stroke (50 ml). Wait 1 minute.
5. Read concentration at the interface of the stained-to-unstained reagent when staining stops

CORRECTION FOR TEMPERATURE, HUMIDITY AND PRESSURE:

Calibration of the Gastec Detector Tube No 31 is based on a tube temperature of 20°C (68°F) and not the temperature of gas being sampled, approximately 50% relative humidity and normal atmospheric pressure. No correction is required for tube temperature of 0–40°C (32°–104°F) and for relative humidity range of 0–100%. To correct for pressure, multiply tube reading by

$$\frac{760}{\text{Atmospheric Pressure (mm)}}$$

CALIBRATION AND ACCURACY:

The Gastec Detector Tube No. 31 is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using cylinder bottle standard gas

DETECTION PRINCIPLE:

Oxygen reacts with titanium trichloride and produces titanium dioxide and hydrogen chloride and produces white color stain

INTERFERENCES:

Ammonia, hydrogen chloride, hydrogen sulfide, sulfur dioxide, nitrogen dioxide, halogens, Carbon dioxide, and Carbon monoxide do not give any effect on tube reading

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI STROKE GAS SAMPLING PUMP.

Manufacturer . Gastec Corporation, Yokohama Japan
86K-31-5

Printed in Japan

GASTEC

CARBON DIOXIDE
EXTRA LOW RANGE TUBE NO. 2LL

The Gastec Detector Tube No 2LL provides a rapid, fully quantitative analysis of the concentration of CARBON DIOXIDE in air with an accuracy tolerance utilizing the Gastec Multi-Stroke Gas Sampling Pump

PERFORMANCE:

Calibration Scale	300 – 5,000 ppm (based on 1 pump stroke)		
Measuring Range	100 – 300 ppm	300 – 5000 ppm	4600 – 11,500 ppm
Number of Pump Strokes	3	1	1/2
Detecting Limit*	30 ppm	—	—
Shelf Life	3 years		
Sampling Time	3 minutes/pump stroke		
Color Change	White – Purple		

*The minimum detectable concentration.

MEASUREMENT PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing toward the pump
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and housing of the pump
4. Pull the handle all the way out until it locks on 1 pump stroke (100 ml) Waiting until staining stops
5. Read concentration at the interface of the stained to-unstained reagent.
6. If the stain length extends over the highest calibration mark, use 1/2 stroke reading (50 ml) and obtain the true concentration by multiplying the tube reading by 2.3
7. If the stain does not attain to the first calibration mark, repeat above sampling procedure 2 more times Obtain true concentration by dividing by 3.
8. To unlock the pump, turn the handle 1/4 turn in either direction

Carbon Dioxide CO_2

Tube Reading (ppm)	300	500	1,000	2,000	3,000	4,000	5,000
1/2 Pump Stroke (50 ml)	—	—	—	4,600	6,900	9,200	11,500

CORRECTION FOR TEMPERATURE, HUMIDITY OR PRESSURE:

Calibration of the Gastec detector tube No 2LL is based on a tube temperature of 20°C (68°F) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperatures of 0° – 40°C (32° – 104°F) and for relative humidity range of 10 – 90% To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:

The Gastec detector tube No. 2LL is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of standard reference of known concentrations and dynamic gas flow system, and wet chemical colorimetric technique (Barium hydroxide-Phenolphtalein method) or gas chromatographic technique

DETECTTON PRINCIPLE:

Carbon dioxide reacts with hydrazine to form carbonic acid monohydrazide, which discolors redox indicator (crystal violet).

$$CO_2 + N_2H_4 \longrightarrow NH_2 \cdot NH \cdot COOH$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Ammonia	Up to 1,000 ppm	No effect	At more than 1 000 ppm gives minus error
Carbon monoxide	Up to 500 ppm	"	
Sulfur dioxide	Up to 30 ppm	"	
Nitrogen dioxide	Up to 30 ppm	"	
Chlorine	Up to 20 ppm	"	

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average by ACGIH (1985): 5,000 ppm (7 – 8 hours)

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI STROKE GAS SAMPLING PUMP.

Manufacturer. Gastec Corporation, Yokohama, Japan
86E-2LL-3

Printed in Japan

GASTEC

CARBON MONOXIDE
EXTRA LOW RANGE DETECTOR TUBE NO. 1LL

The Gastec Detector tube No 1LL provides a rapid, quantitative analysis of the concentration of CARBON MONOXIDE in air with a maximum accuracy of ±25% using the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	5–50 ppm (based on 2 pump strokes)
Measuring Range	5–50 ppm
Number of Pump Stroke	2
Correction Factor	Tube reading × 1
Detecting Limit*	1 ppm
Sampling Time	2 minutes per pump stroke
Color Change	Yellow – Brown
Shelf Life	2 years

*Maximum detectable concentration.

MEASUREMENT PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and housing of the pump.
4. Pull the handle all the way out until it locks on 1pump stroke (100 ml). Wait until staining stops. Repeat this sampling procedure one more time without removing the tube. For 2 pump stroke (200 ml) sampling, the handle must be turned 1/4 turn in either direction to unlock the pump so the handle can be returned to the starting position.
5. Read concentration at the interface of the stained-to-unstained reagent when staining stops after completion of 2 pump stroke (200 ml) sampling.

CORRECTION FOR TEMPERATURE, HUMIDITY OR PRESSURE:

Calibration of the Gastec detector tube No 1LL is based on a tube temperature of 20°C (68°F) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperatures of 0-40°C (32°F–104°F) and for relative humidity range of 20–90%. To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:

The Gastec detector tube No 1LL is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of standard reference gas of known concentrations and dynamic gas flow system, and nondispersive infrared adsorption (NDIR) or gas chromatographic technique.

DETECTION PRINCIPLE:

Carbon monoxide reduces potassium palladosulfite to liberate metallic palladium which produces a brown stain.

$$CO + K_2Pd(SO_3)_2 \longrightarrow K_2SO_3 + Pd + CO_2 + SO_2$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Carbon disulfide	1/50 of CO conc.	Plus error	Also produces similar stain by itself
Acetylene	1/50 of CO conc.	"	"
Hydrogen sulfide	1/50 of CO conc.	"	"
Mercaptans	1/50 of CO conc.	"	"
Phosphine	1/10 of CO conc.	"	"
Phosgene	1/10 of CO conc.		"
Sulfur dioxide	1/10 of CO conc.	"	No stain by itself
Ethylene	Up to 0.1%	No effect	
Hydrogen	Up to 0.2%	"	
Nitrogen dioxide		"	

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average by ACGIH (1980). 50 ppm (7–8 hours)

Threshold Limit Value-Short Term Exposure limit by ACGIH (1980) 400 ppm (15 minutes)

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI-STROKE GAS SAMPLING PUMP.

Manufacturer: Gastec Corporation, Yokohama, Japan
86K-1LL-5

Printed in Japan

GASTEC

PHOSPHINE LOW RANGE DETECTOR TUBE NO. 7L

The Gastec Detector Tube No. 7L provides a rapid, fully quantitative analysis of the concentration of PHOSPHINE in air with a minimum accuracy of ±25% at 1, 2 and 5 times TLV or ±35% at 1/2 TLV utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	0.3—5 ppm (based on 5 pump strokes)	
Color Change	Dull Yellow—Purple	
Shell Life	3 years	
Measuring Range	0.15—2.5 ppm	0.3—5 ppm
Detecting Limit *	0.04 ppm	0.08 ppm
Pump Strokes	10	5
Sampling Time	1 minute per pump stroke	

* The minimum detectable concentration

MEASURING PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the guide marks on the shaft and housing of the pump.
4. Pull the handle all the way out until it locks on 1 pump stroke (100 ml). Wait until staining stops.
5. Repeat this sampling procedure four (4) more times without removing the tube. For repeated pump stroke sampling, the handle must be 1/4 turn in either direction to unlock the pump so the handle can be returned to the starting position.
6. Read concentration at the interface of the stained-to-unstained reagent when staining stops after completion of 5 pump stroke (500 ml) sampling.
7. If the discoloration is before the first calibration mark (0.3 ppm), repeat the above sampling procedure five (5) more times without removing the tube. Obtain true concentration by dividing the tube reading by 2.

CORRECTION FOR TEMPERATURE HUUIDITY OR PRESSURE:
Calibration of the Gastec detector tube No. 7L is based on a tube temperature of 20℃ (68℉) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperature of 0°—40℃ (32°—104℉) and for relative humidity range of 20—90%. To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:
The Gastec detector tube No 7L is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combination of dynamic dilution board system and gas chromatographic technique.

DETECTION PRINCIPLE:
PH_3 + Gold compound ——→ Colloidal gold

DANGEROUS AND HAZARDOUS PROPERTIES:
Threshold Limit Value-Time Weighted Average by ACGIH (1984). 0.3 ppm (7—8 hours)
Threshold Limit Value-Short Term Exposure Limit by ACGIH (1984): 1 ppm (15 minutes)

INTERFERENCES:

Interferent	Concentration	Result	Comment
Arsine		Plus error	Produce similar stain by it self
Hydrogen Chloride		"	"
Hydrogen Selenide		"	"
Hydrogen Sulfide		"	"
Mercaptans		"	"

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI-STROKE GAS SAMPLING PUMP.

Manufacturer Gastec Corporation Yokohama, Japan
85A-7L-2
Printed in Japan

GASTEC

N-HEXANE LOW RANGE DETECTOR TUBE NO. 102L

The Gastec Detector Tube No. 102L provides a rapid, fully quantitative analysis of the concentration of n-HEXANE in air with a minimum accuracy of ±25% at 1, 2, and 5 times TLV or ±35% at 1/2 TLV utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	50–1200 ppm (based on 1 pump stroke)		
Measuring Range	10–50 ppm	50–1200 ppm	1200–2640 ppm
Number of Pump Stroke	5	1	1/2
Correction Factor	Tube reading ÷ 5	Tube reading × 1	Tube Reading × 2.2
Detecting Limit*	1 ppm	—	—
Sampling Time	2 minutes per pump stroke		
Color Change	Orange – Brownish Green		
Shelf Life	3 years		

* Minimum detectable concentration.

MEASUREMENT PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing towards the pump.
3. Make certain the pump handle is all the way in. Align the guide marks on the shaft and housing of the pump.
4. Pull the handle all the way out until it locks on 1 pump stroke (100 ml). Wait until staining stops.
5. Read concentration at the interface of the stained-to-unstained reagent. If the stain produces channeling, take the stain length of farthest extended and least extended along the tube's longitudinal axis and read the concentration at the mean value.
6. When the measuring concentration is over the 1200 ppm, or if the stain length extends over the highest calibration mark by 1 pump stroke sampling, use 1/2 stroke sampling (50 ml), in which case the true concentration is obtained by multiplying the tube reading by 2.2.
7. For more accurate measurement of such a lower concentration as less than 50 ppm use 5 pump stroke sampling, in this case the true concentration is obtained by dividing the tube reading by 5.
8. To unlock the pump, turn the handle by making 1/4 turn in either direction.

CORRECTION FOR TEMPERATURE, HUMIDITY AND PRESSURE:

Calibration of the Gastec Detector Tube No. 102L is based on a tube temperature of 20°C (68°F) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No temperature correction is required for tube temperatures of 0°–40°C (32°–104°F). Moisture in the sample is controlled in the prelayer, therefore, does not affect accurate tube readings. Tube reading is proportional to absolute pressure. To correct the tube reading for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:

The Gastec detector tube No 102L is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of dynamic diffusion tube method and gas chromatographic technique.

DETECTION PRINCIPLE:

n-Hexane reduces potassium dichromate to form chromic sulfate, which is green in color.

$$C_6H_{14} + K_2Cr_2O_7 + H_2O \longrightarrow Cr_2(SO_4)_3$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Other organic vapors except halogenated hydrocarbons			Produce similar stain by themselves

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average (TLV-TWA) by ACGIH (1986): 50 ppm (7–8 hours)

Flammable Limit: 1.2–7.5%

APPLICATION FOR OTHER GASES:

The detector tube No 102L can also be used for the measurement of tert-Butyl Alcohol in air. Concentration of the substance can be obtained from the table below.

tert-Butyl Alcohol $(CH_3)_3COH$

Tube Reading 102L	50	100	200	400	600	800	1000	1200
$(CH_3)_3COH$ ppm 2 pump strokes	1000	1700	2700	4100	5400	6600	7700	8900

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI STROKE GAS SAMPLING PUMP

Manufacturer: Gastec Corporation Yokohama, Japan
86G-102L-5

Printed in Japan

Appendix C. ORNL Report: Characterization of Inhalation Exposure Atmospheres at the Denver Wildlife Research Center (August 17-18, 1987).

USDA/DWRC/ORNL Topical Report

Characterization of Inhalation Exposure Atmospheres at the Denver Wildlife Research Center August 17-18, 1987

USDA/APHIS Funding No. 87-74-01

DOE Interagency Agreement No. 1578-1578-A1

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R. L. Moody
R. A. Jenkins

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Oak Ridge, TN 37831-6120

Report Preparation Date: November 28, 1988

Note: This report contains an Appendix A which should not be confused with the main Appendices of the current report.

Characterization of Inhalation Exposure Atmospheres

Denver Wildlife Research Center

USDA-APHIS

August 17-18,1987

J. H. Moneyhun, R. L. Moody and R. A. Jenkins

Executive Summary

A working site visit was made to the Denver Wildlife Research Center (DWRC-USDA/APHIS) in order to characterize the inhalation exposure atmosphere being generated by the continuous combustion of hexane-softened red phosphorus-butyl rubber. Aerosol levels at the extremes of the concentration ranges used in the bioassays (1 and 6 mg/L) were investigated. Samples were acquired and returned to Oak Ridge National Laboratory for analysis.

Data indicated that the phosphoric acid produced comprised about 65-80% of the particulate mass present in the aerosol. At higher aerosol concentration, the particulates are probably not at equilibrium with the atmospheric moisture. Levels of total organic carbon in the aerosol were less than 10 $\mu g/L$ or no more than 3 $\mu g/mg$ H_3PO_4. Particle sizes for both the high and low aerosol concentration runs were less than 1 μm mass median diameter and therefore, well within the respirable range. Although detector tube analysis of residual hexane in the exposure atmosphere by DWRC personnel had suggested that the hexane levels were 50 ppm or more, exhaustive thermal desorption GC/MS analysis of vapor samples indicated levels of 5 ppm or less. This is in much better agreement with expected levels. CO_2 levels reached maxima of 760 ppm, indicating rather complete combustion of the residual organics. Carbon monoxide levels reached 50 ppm, but only for the worst-case-scenario 6 mg/L. The data indicated that, for the most part, the atmosphere generated at DWRC is identical to that generated for another inhalation study conducted elsewhere, and to that generated during characterization studies at ORNL.

Introduction

The Denver Wildlife Research Center (DWRC) (U.S. Department of Agriculture Animal and Plant Health Inspection Service), in agreement with the U.S. Army Medical Research and Development Laboratory, has been conducting an inhalation toxicology study whereby two species of wildlife (ie. rock doves and prairie dogs) are exposed to an aerosol generated by burning a mixture of red phosphorus and butyl rubber. This formulation is employed by the military as one of their battlefield obscurants. Burning the phosphorus in air produces phosphorus pentoxide.

When contacting atmospheric moisture, the pentoxide hydrolyzes to form a phosphoric acid aerosol comprised predominantly of a mixture of polyphosphoric acids. In the field, the resulting aerosol forms a white cloud that is an effective visual obscurant. The purpose of the agreement is to determine the extent of adverse behavioral and toxicological effects on wild vertebrates which may be exposed to the smoke during military training exercises. The Analytical Chemistry Division of Oak Ridge National Laboratory (ORNL) maintained a support role for the investigation by supplying smoke generation hardware and methodology, as well as physical and chemical documentation of the exposure atmospheres.

The animals are exposed to a smoke aerosol generated using an extrusion/burn apparatus developed at Oak Ridge National Laboratory and previously used in a similar study at the Illinois Institute of Technology Research Institute (IITRI)[1]. In this system, the red phosphorus/butyl rubber formulation (RPBR) is softened batchwise with hexane vapors and loaded into billets. The RPBR is extruded from the billet in a ribbon by an hydraulic system, and subsequently is burned to form the aerosol. The aerosol concentration is ultimately controlled by driving the hydraulic system at a variety of selected rates with a metering pump to extrude the RPBR in a continuous manner.

Differences in altitude between the DWRC and IITRI sites results in an atmospheric pressure differential of approximately 80 mm Hg, (atmospheric pressure at Denver averages near 660 mm Hg while in Chicago - and Oak Ridge - the average atmospheric pressure is approximately 740 mm Hg). This causes a diminished oxygen content in Denver, which could potentially alter the burn characteristics and thereby the composition of the exposure atmosphere. In order to document that composition, a working site visit was made to DWRC on August 17-18, 1987 by two ORNL staff members (R.L. Moody and J.H. Moneyhun).

The purpose of the visit was to acquire samples of the exposure atmosphere for detailed chemical analysis at ORNL. Results are summarized on the following pages.

Aerosol and Phosphoric Acid Concentrations

In order to establish concentration levels, filter samples were acquired by sampling the chamber atmosphere at 1.5 L/min for 10 minutes each during the low concentration experiments (ca. 1 mg/L) and 5 minutes during the high concentration RPBR burns (ca 6 mg/L). These filters were weighed prior to and following sampling to establish total mass concentrations. Since the aerosol is a solution of phosphoric acid in water, the filters were subsequently eluted with water and an aliquot of the eluate subsequently analyzed for total phosphate. Phosphate was determined by hydrolyzing all the polyphosphoric acids to orthophosphoric acid and reacting with molybdic acid to form a molybdenum blue complex that was measured spectrophotometrically[1]. No effort was made to determine the distribution of individual polyphosphoric acids, because the toxicological significance of the speciation was believed to be minimal.

Phosphate analyses are also routinely performed at DWRC by titration of the phosphoric acid. One sample from each run was analyzed using this technique by the DWRC personnel. Table 1 lists the mass concentration, based upon filter weights, and the phosphoric acid concentration for these samples. In some cases, the samples analyzed for phosphoric acid by titration at the Denver site yielded slightly different results for phosphoric acid than those analyzed at ORNL by the spectrophotometric procedure. However, in all cases, the differences were not large and judged not to be important.

After a run was started, no significant effort was made to adjust the concentration to a specific level as would be done during an actual exposure experiment. However, the concentrations obtained are reasonably consistent with the desired levels of 1 mg/L (g/m^3) for the low concentration and 6 mg/L (g/m^3) for the high concentration. The low concentration in Run No. 3, Sample No. 3 resulted from the loss of the flame caused by a break in the RPBR ribbon behind the flame. Concentration in the chamber dropped before the burn was restarted. Once the burning was restarted, the concentration returned to a nominal value (Run 3/Sample 4).

Air supply to the generator system at DWRC is temperature and humidity controlled, with water vapor being added to the normally dry ambient air. Humidity measurements are made before a burn starts and at the end of a run before stopping the generator. The water vapor absorbed by the phosphoric acid aerosol is a function of humidity and the time between aerosol formation and collection. Under conditions in which the phosphoric acid droplets have not had the time to achieve complete equilibrium with their surroundings (such as those which exist inside the chamber) and fixed water vapor concentration, the greater the amount of H_3PO_4 is present, the greater the fraction of the aerosol mass which will be comprised of H_3PO_4. The phosphoric acid effectively scavenges water vapor from the air. The higher the acid concentration, the lower the fraction of water in any one droplet. This type of phenomenon is portrayed in Figure 1. The line represents literature values[2], calculated from vapor pressure measurements over phosphoric acid solutions. The stars represent data generated at ORNL[3], at a constant aerosol concentration while varying the humidity in the incoming air. In general, the data agrees with the literature values. The low aerosol concentration data at DWRC is fairly close to the expected values. The higher concentration mean values at DWRC (as measured by the molybdic acid method at ORNL) are all about 10-15% higher than the expected values, indicating that equilibrium between the particulates and the ambient humidity has not been reached at sampling time. (Interestingly, high concentration H_3PO_4 fraction values determined at DWRC using the titration method were somewhat closer to the expected values than the ORNL determined values). Similar results have been observed for burns at ORNL, where humidity was held constant and aerosol concentration was varied.

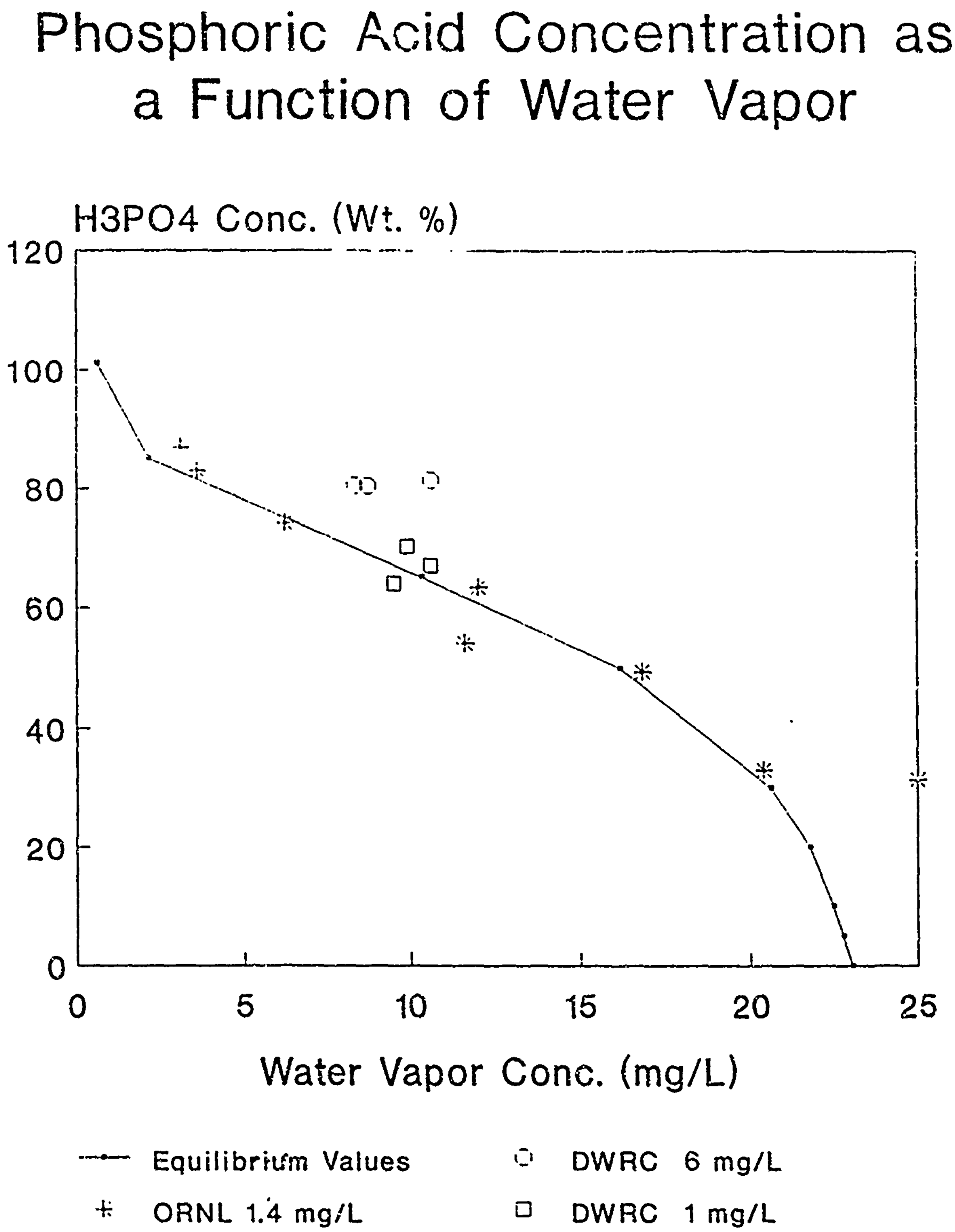
Phosphoric Acid Concentration as a Function of Water Vapor
H3PO4 Conc. (Wt. %)
120
100
80
60
40
20
0
0
5
10
15
20
25
Water Vapor Conc. (mg/L)
Equilibrium Values
ORNL 1.4 mg/L
DWRC 6 mg/L
DWRC 1 mg/L

Figure 1.

Table 1. Inhalation Exposure Atmosphere Mass and Phosphoric Acid Concentrations

Sample Code Phase(%)	Sample Volume (Liters)	Sample Weight (mg.)	Aerosol Mass Concentration (mg/L)	H_3PO_4 mg. per sample	H_3PO_4 Fraction of Particulate
Low Concentration					
Run 1/1	16.7	20.3	1.21	14.65	72.2
Run 1/2	16.7	19.5	1.17	14.32	73.4
Run 1/3[a]	16.7	19.4	1.16	12.61	65.0
Run 3/1	16.7	15.3	.92	10.45	68.3
Run 3/2	16.7	15.0	.90	9.83	65.5
Run 3/3*	16.7	4.3	.26	2.97	69.0
Run 3/4[a]	16.7	16.3	.98	10.56	64.8
Run 5/1	16.7	13.8	.83	9.5	68.6
Run 5/2	16.7	16.7	1.00	9.8	58.5
Run 5/3[a]	16.7	17.9	1.07	11.54	64.5
High Concentration					
Run 2/1	8.35	55.5	6.65	46.77	84.3
Run 2/2	8.35	54.7	6.55	44.65	81.6
Run 2/3[a]	8.35	52.7	6.31	39.63	75.2
Run 4/1	8.35	50.7	6.07	41.9	82.7
Run 4/2	8.35	50.2	6.01	41.5	82.6
Run 4/3[a]	8.35	55.3	6.62	41.92	75.8
Run 6/1	8.35	57.5	6.88	48.8	84.8
Run 6/2	8.35	55.2	6.61	45.9	83.2
Run 6/3[a]	8.35	51.6	6.18	39.18	75.9

[a] Samples from these runs analyzed phosphoric acid by titration by Denver personnel.

*Low concentration in this sample resulted from temporary loss of flame. See text.

Total Organic Carbon

Since the "fuel" for the generator contains butyl rubber and is softened by the addition of hexane, organic species would be expected to be formed during combustion. A fraction of those may be associated with the particulates. Total organic carbon was determined in the particulate phase by collecting samples in an impinger filled with water. The impinger was experimentally found to collect over 97% of the aerosol, based on filter samples placed downstream of the impinger. The particulates were oxidized in order to convert organic compounds to carbon dioxide and measured on a commercially available TOC analyzer.

Table 2 lists results of these determinations. The TOC values averaged 1.3μg/L for the low concentration runs, and 5.3μg/L for the higher concentration runs, and are higher than those found in the ORNL study by a factor of 10. However, similar samples taken at the IITRI site were found to have the same TOC levels as those taken at DWRC. Overall, the levels of TOC are still less than 10 μg/L of aerosol. If all of the TOC were present as unreacted hexane, the hexane level in the particulates would be only about 3 ppm.

Samples taken for TOC determination from burns conducted at ORNL were sampled at a much higher sampling rate and for much longer duration, collecting total sample weights approximately 20 times that collected at IITRI or Denver. This does not explain the differences noted, but is the only difference between the sampling procedures.

Table 2. Total Organic Carbon (TOC) Present in Phosphoric Acid Aerosol

Sample Code	TOC (μg/L Aerosol) in particulates	TOC μg/mg H_3PO_4
Background 1	0.8	
Background 2	0.8	
Low Aerosol Concentration		
Run 1	0.9	1.1
Run 3	1.7	2.8
Run 5	1.4	2.3
Mean	1.3	2.1
High Aerosol Concentration		
Run 2/ 1	5.1	1.0
Run 2/ 2	4.6	.9
Run 4/ 1	8.3	1.7
Run 4/ 2	4.3	.9
Run 6/ 1	6.9	1.3
Run 6/ 2	2.6	.5
Mean	5.3	1.1

Aerosol Particle Size Distributions

Cascade impactor samples were taken for particle size determination. Two impactors were used, each sampling at 1 L/min[4]. Duplicate samples were taken from each burn, one with each impactor. The stages were analyzed for

H_3PO_4 and particle size determinations were made assuming a log normal distribution. The data from duplicates were plotted on a single graph form and used to construct the log normal plot. For these samples, since the phosphoric acid concentration was known from analysis of filter samples, density of the aerosolized material could be determined and corrections were made for densities. The resulting particle sizes are therefore reported as Stokes or physical diameters rather than as aerodynamic diameters. Aerodynamic diameters, assuming densities of 1 g/cc, would be approximately 30% greater than the Stokes diameters. The density of phosphoric acid solutions of these concentrations is approximately 1.5 -1.7 g/cc. The particle size distributions are reported in Table 3.

Table 3. Cascade Impactor Samples - Particle Size Determination

Low Aerosol Concentration - 1 mg/L

	MMD* uM	σG**
Run 1	0.67	1.41
Run 3	0.60	1.47
Run 5	0.55	1.48
Mean	0.61	1.45

High Aerosol Concentration - 6 mg/L

	MMD* uM	σG**
Run 2	0.96	1.50
Run 4	0.95	1.48
Run 6	0.92	1.51
Mean	0.94	1.50

* Mass Median Diameter (Stokes Diameter)
** Geometric Standard Deviation

At both the high and low aerosol concentrations, the mass median diameters were less than 1μM in diameter and are well within a respirable range. The particle size of the higher concentration is slightly larger than has been observed at ORNL but probably results from increased residence time of the aerosol in the chamber. That is, to attain the high concentration of 6 mg/L, it was necessary to set the air flow at only 250 L/min through the chamber. (Normal flow rate is 500 L/min). This lower air flow causes a longer residence time in the chamber and should result in larger particle size as the phosphoric acid has more time to scavenge water vapor, and coagulate with other droplets. In chambers where there is little turbulence (nearly laminar flow), differences in particle size are detectable between samples taken at the top and bottom of the chamber[4]. This type of phenomenon supports the contention that aerosol droplet size can grow under these flow conditions.

Vapor Phase Components

There is always the possibility that in burning the red phosphorus formulation, toxic organic vapors may be formed either from the butyl rubber of the formulation or from the hexane used to soften the material in order that it may be extruded. Also, carbon monoxide and carbon dioxide are formed from the combustion of the organic materials. Although the aerosol is formed in an oxidizing medium, there has been concern that phosphine (PH_3 - a highly reduced compound) could either be formed or be present in the fuel and released into the chamber. DWRC personnel have routinely used Gastech detector tubes (chemically reactive traps yielding color changes to target components) to monitor for these components. Components measured in this manner were phosphine, oxygen, carbon dioxide, carbon monoxide, and hexane.

To determine hexane and other organic components in the vapor phase on this sampling effort, triple adsorbent traps were used to sample the vapor from the chamber. These vapor traps were 1/4 inch od. stainless steel tubes 6 inches in length, and packed with sections of Tenax followed by Carbotrap and then Ambersorb XE-340. These traps have been used successfully in our laboratory to collect organic vapors[5]. The trap is desorbed thermally into an analytical system such as a gas chromatograph, and in this case to a gas chromatograph followed by a mass spectrometer GC/MS. Quantitative GC/MS analysis indicated that only hexane and methylcyclopentane were present in measurable concentrations. The values are reported in Table 4.

Hexane levels were less than 1 ppm at the low aerosol concentrations and approximately 5 ppm for the higher concentrations. This is approximately 15 μg/L of aerosol and is well below the OSHA limit of 180 μg/L for an 8 hour time weighted average. The levels found at DWRC are similar to those determined at ORNL, where hexane levels of approximately 1 ppm per mg/L of aerosol were observed.

These values as determined by GC/MS are as much as a factor of twenty below values measured by the Gastech sampling systems used by DWRC personnel. To verify the efficacy of our traps for the determination of hexane vapor, a series of experiments were performed at ORNL. Hexane vapors were generated, using a diffusion tube. The vapor was diluted with air, and passed into a chamber. Concentration was determined by weight change in the diffusion tube and a carefully measured dilution air flow. The concentration was also monitored continuously using a total hydrocarbon monitor (flame ionization detector). Triple sorbent trap samples were taken of the test chamber atmosphere and analyzed by GC/MS in a manner identical to that of the field samples. Their results are shown in Table 5.

Table 4. Vapor Phase Organic Concentrations
RPBR Exposure Atmospheres at DWRC

Sample Type	Triple Sorbent - GC/MS Analysis[a]		Detector Tube Analysis[b]
	Hexane (ppm)	Methyl Cyclopentane (ppm)	Hexane (ppm)
Chamber Blank[c]	0.0	ND	ND
Trip Blank[d]	0.0	ND	-
Low Aerosol Concentration			
Burn 1	0.31	0.016	50
Burn 3	0.05	NQ	50
Burn 5	ND	NQ	ND
High Aerosol Concentration			
Burn 2/1	3.1	0.36	60
Burn 2/2	5.4	NQ	
Burn 4/1	4.2	0.22	100
Burn 4/2	4.4	NQ	
Burn 6/1	2.6	0.28	80
Burn 6/2	2.4	NQ	

ND - None Detected
NQ - Not Quantified
[a] Determined at ORNL, using thermal desorption GC/MS
[b] Determined at DWRC, using detector tubes

Table 5. Comparison of Predicted Vs. Observed Hexane Vapor Levels
Using Triple Sorbent Trap - GC/MS Sampling/Analysis Procedure

Sample Code	Sampling Duration, (min.)	Flow Rate, (L/min.)	Hexane Concentration[a] μg/L	Trap Loading of Hexane, μg Predicted	Trap Loading of Hexane, μg Observed
Blank #1	10	0.53	0	0	0
Blank #2	10	0.53	0	0	2.5
TS 1	1	0.53	8.84	4.7	5.0
TS 2	1	0.53	8.60	4.60	5.4
TS 3	2	0.53	8.48	9.0	8.4
TS 6	4	0.53	9.88	21.1	18.2
TS 7	6	0.53	9.49	30.4	29.0

[a] Based on weight reduction from diffusion tube

Good agreement is shown between the theoretical loadings of the traps and those actually observed. We are confident that the hexane values determined by this technique are valid. We make no attempt to explain the higher values determined by the Gastech detector tube procedure except to state that the RPBR aerosol is a rather complex system and the sampling tubes are designed for atmospheres contaminated with a limited number of components. It would seem possible that the high concentrations of phosphoric acid mist could somehow interfere with the chemical reactions in the detector tubes.

Further support for the contention that the hexane concentration is not as high as measured by the detector tube procedure is presented in calculations in Appendix A. A calculation is performed showing the theoretical upper bound of hexane vapor that could be present in the exposure atmosphere if none of the hexane used to soften the RPBR is burned. That value of 40 ppm for the high concentration burns is below the OSHA limits for human exposure (50 ppm) and the values determined by the Gastech procedures at Denver.

Additionally, a calculation of carbon dioxide (CO_2) produced from burning the butyl rubber and the hexane that would be in the softened material, as burned, is also in the appendix. The formulation is a blend of 95% phosphorus and 5% butyl rubber. Hexane is added to the blend by vapor absorption to a concentration of 7.5 % by weight. By calculating the volume of CO_2 that will be produced from the quantity of softened RPBR needed to produce 6 mg/L of aerosol in the chamber, it is shown that if all the hexane and the butyl rubber is burned, ca. 0.39 cc of CO_2/L is formed. This is 390 ppm carbon dioxide in the chamber. Based upon the gas chromatographic analyses of samples returned to ORNL, 350 ppm CO_2 above background was found in the chamber. This, along with the concentrations of CO found and the measured concentrations of hexane found by the vapor trap procedure, a rather good material balance is achieved, suggesting near complete combustion of the hexane and butyl rubber.

To determine other gas phase components, three types of samples were taken. Draeger type indicating sampling tubes were used to sample directly from the chamber. Phosphine was determined in this manner. Filtered atmosphere samples for CO and CO_2 were withdrawn from the chamber using small diaphragm pumps and collected in Tedlar gas sampling bags. CO was measured by drawing the sample from the bag through an Ecolizer and a Dreager tube. CO_2 was also measured from the Tedlar bag using a Dreager tube. Additionally, a sample was transferred from the bag to a glass gas sampling bulb and brought to the Oak Ridge National Laboratory for CO_2 analysis. CO_2 was analyzed by gas chromatography[6]. Results of these analyses are shown in Table 6.

Carbon dioxide levels are approximately twice that of the ambient concentrations for the high concentration runs. Previous measurements at IITRI and ORNL are comparable to these results. It is interesting to note that the carbon dioxide contribution from test animals in the exposure chamber (40 rats) at IITRI was similar in magnitude to the amount contributed by the burning of the RPBR.

Table 6. Aerosol Concentrations of Selected Vapor Phase Constituents As Determined by Multiple Analytical Methods

	Carbon Dioxide, ppm		Carbon Monoxide, ppm		Phosine, ppm
	GC	Draeger Tube	Ecolyzer	Draeger Tube	Draeger Tube
Chamber Blank	394[a]	300	ND	-	ND
Low Concentration					
Run 1	506	400	31	20	ND
Run 3	478	300	16	10	ND
Run 5	469	300	26	13	ND
High Concentration					
Run 2	722	400	50	30	<0.1
Run 4	750	500	54	32	<0.1
Run 6	759	500	53	35	NQ

[a] Average of 5 determinations
ND = None Detected
NQ = Not Quantified

Carbon monoxide levels for the high concentration burns are at the OSHA limit of 50 ppm for an eight (8) hour exposure. This is somewhat higher than observed in burns conducted at ORNL. Aerosol concentrations at ORNL ranging from 2mg/L to 5 mg/L resulted in carbon monoxide concentrations of only ca. 25 ppm. There was no increase in carbon monoxide at the higher burns, as was the case at the DWRC. Burns at IITRI were conducted at much lower aerosol concentrations (less than 1.25 mg/L) and carbon monoxide concentrations ranged from 8 to 24 ppm for all burns. The higher carbon monoxide concentration at DWRC could be caused by the lower flow rates of air across the point at which the RPBR is burning. As stated previously, in order to attain the 6 mg/L aerosol concentration, it was necessary to lower the dilution air flow rate. This dilution air is drawn through the burn chamber. With the lower flow rate, less turbulence would exist, and oxygen may become depleted at the point of combustion. This could result in less complete combustion and a higher resulting CO concentration in the chamber.

Phosphine determinations using the Dreagers tube were inconclusive. Only slight discoloration at the base of the sampling tube was observed. Estimates of concentrations were based upon the lower detection limit of the sample tube. A more precise gas chromatographic technique used at ORNL could establish no more conclusive values. The OSHA established limit of 0.3 ppm would have been detected by either of the two methods.

Conclusions

Little difference was observed between the exposure atmosphere generated at DWRC and that generated at ORNL. Although the hexane vapor concentration as determined by the detector tubes was found to be at the OSHA exposure level of 50 ppm, the levels as determined by the much more accurate GC/MS method were well below that limit. The hexane tests performed at ORNL by generating a known hexane vapor concentration and sampling with the triple sorbent traps verify that the method is accurate at the levels found. Additionally, calculations indicate that even if none of the hexane was consumed by combustion, the concentration of hexane could not reach the levels measured by the Gastech system.

The TOC concentration associated with the particulates is higher than that reported for burns at ORNL, but is the same level as that determined at IITRI. The TOC concentration is only a few micrograms per liter in the higher concentration atmospheres.

Carbon monoxide in the high (6 mg/L) concentration atmospheres is above levels determined at ORNL and is at the OSHA limit of 50 ppm. Other constituents are very similar in concentration to that found in the original characterization efforts.

We consider the product generated by the extrusion burn method to be representative of that product produced in the field. Differences observed between the RPBR aerosol as generated at DWRC and that at ORNL are minimal and not sufficiently great to preclude the use of the aerosol for the toxicological study. Most differences appear to be due to air flow or oxygen concentration. Such differences would be encountered to some extent in a field situation. In the field, some of the aerosol is produced while the RPBR pellets are flying through the air. In such a case, oxygen depletion at the combustion site would be minimized. However, some of the aerosol is generated while the pellets are lying on the ground and burning. In this situation, occasional oxygen depletion near the point of burning would be expected.

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APPENDIX A

CALCULATION OF MAXIMUM EXPECTED HEXANE AND CARBON DIOXIDE LEVELS IN EXPOSURE ATMOSPHERE

Calculation of Maximum Hexane Vapor Content of Exposure Atmosphere

Average H_3PO_4 fraction of total aerosol mass in high concentrations burns (6mg/liter) = 80.7%

At 6 mg/liter aerosol concentration, then H_3PO_4 level = 4.84 mg/L as H_3PO_4

$[P]/[H_3PO_4]$ = 31/98 = .316 mg P/mg H_3PO_4

The RPBR blend is 95% P, 5% butyl rubber

The RPBR blend is softened with 7.5% hexane, by weight

Thus,

mg P/ liter aerosol = 1.53 mg

mg BR/liter aerosol = .081 mg

mg hexane/liter aerosol = .121 mg

hexane vapor @ STP = 22.4 L/86 grams

= .26 ml/mg hexane

For atmospheric conditions at Denver, this would expand by

$\frac{760\text{mm Hg} \times 294°\text{K}}{660\text{mm Hg} \times 273°\text{K}}$, or 0.33 ml/mg.

Then .121 mg hexane x .33 ml/mg hexane

= .040 ml hexane vapor or

= 40 ppm hexane if none burned

Calculation of Maximum Volume of CO_2 Formed
from Hexane and Butyl Rubber Combustion

hexane + oxygen --> Carbon dioxide + water

At a 6 mg/L aerosol concentration, there would be CO_2 contributions from 0.121 mg hexane and 0.081 mg butyl rubber, per liter of aerosol.

2 C_6H_{14}	+	19 O_2	-->	12 CO_2	+	14 H_2O
1 mg			yields	3.06 mg		
.121 mg			yields	.370 mg		

butyl rubber + oxygen --> Carbon Dioxide + water

[C_4H_8]	+	6 O_2	-->	4 CO_2	+	4H_2O
1 mg			yields	3.14 mg		
.081 mg			yields	.254 mg		

Maximum CO_2 from hexane and butyl rubber = .624 mg per liter aerosol.

44 grams CO_2 = 22.4 liters @ STP

0.624 mg CO_2 = 0.318 ml CO_2 @ STP, or 0.394 mL CO_2 per liter aerosol at Denver atmospheric conditions

Thus, maximum CO_2 level attainable would be 394 ppm above background, or 788 ppm

Appendix D. Standard Gross Necropsy Forms for Prairie Dogs and Rock Doves

Task 2 Effective Smoke Concentration Range Finding STUDY I.D. #DOD-T

Species Prairie Dog______ Exposure Concentration______
Animal Number______ Number of 1 hr Exposure Days______
Sex______
- = Normal
+ = Abnormal

Circle Correct Description

1. Nasal Passages
   (a) excess phlem/fluid: + -
   (b) color: normal red grey-blue black
2. Trachea
   (a) ulceration: + -
   (b) exudate: + -
   (c) bleeding: + -
   (d) scarring: + -
3. Larynx
   (a) color: normal red grey-blue black
   (b) texture: + -
   (c) lesions: + -
   (d) exudate: + -
      (1) amount/texture (optional)______
4. Epiglottis
   (a) color: normal red grey-blue black
   (b) texture: normal softened hardened
   (c) size: + -
5. Bronchi
   (a) color: normal red grey-blue black
   (b) fluid: + -
   (c) lesions: + -
6. Lungs
   (a) fluid: + -
   (b) edema: + -
   (c) hemorrhages + -
      (1) location, extent, number of clots (optional)______
7. Heart
   (a) color: normal red grey-blue black
   (b) size: + -
8. Liver
   (a) color: normal red light dark
   (b) texture: + -
   (c) size: + -
9. Spleen
   (a) color: + -
   (b) size: + -
10. Kidney
   (a) color: + -
   (b) size: + -

______
(signature of Certified D.V.M.)

______
(date)

Task 2 Effective Smoke Concentration Range Finding STUDY I.D. #DOD-1

Species Rock Dove ____________ Exposure Concentration ____________
Animal Number ____________ Number of 1 hr Exposure Days ____________
Sex Cloacal exam ____________ - = Normal
Sex Necropsy exam ____________ + = Abnormal Body Weight ________g

Circle Correct Description

*1. Nasal Passages
- (a) excess phlem/fluid: + -
- (b) color: normal red grey-blue black

*2. Trachea
- (a) ulceration: + -
- (b) exudate: + -
- (c) bleeding: + -
- (d) scarring: + -

*3. Larynx
- (a) color: normal red grey-blue black
- (b) texture: + -
- (c) lesions: + -
- (d) exudate: + -
  - (1) amount/texture (optional) ____________

*4. Epiglottis
- (a) color: normal red grey-blue black
- (b) texture: normal softened hardened
- (c) size: + -

*5. Bronchi
- (a) color: normal red grey-blue black
- (b) fluid: + -
- (c) lesions: + -

*6. Lungs
- (a) fluid: + -
- (b) edema: + -
- (c) hemorrhages + -
  - (1) location, extent, number of clots (optional) ____________

7. Heart
- (a) color: normal red grey-blue black
- (b) size: + -

*8. Liver
- (a) color: normal red light dark
- (b) texture: + -
- (c) size: + -

9. Spleen
- (a) color: + -
- (b) size: + -

10. Kidney
- (a) color: + -
- (b) size: + -

____
(signature of Certified D.V.M.)

____
(date)

Appendix E. Prairie Dog Symptom Counts

Frequency table of observed symptom categories over 7-day blocks for each tested prairie dog group exposed to four levels of red phosphorus/butyl rubber smoke for one-to-four daily exposure sessions.

RP/BR Target Concentration	0.0 mg/l			2.0 mg/l			4.0 mg/l			6.0 mg/l		
Successive 7-Session Blocks	Pre-[a]	Post 1[b]	Post 2[c]	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2
ONE RP/BR SMOKE EXPOSURE DAY												
Body posture (rest)	0	1	0	0	0	0	0	0	0	2	1	4
Body posture (exercise)	0	0	0	0	0	0	0	0	0	1	0	0
Respiratory congestion (rest)	0	0	0	0	0	1	0	1	0	0	1	3
Respiratory congestion (exercise)	0	0	0	0	0	1	0	1	1	0	2	4
Ungroomed coat	2	0	0	0	0	1	1	0	1	0	0	4
Aggressive responses	2	0	0	0	1	0	0	2	0	4	4	1
Vocalizations	25	21	13	23	8	7	23	14	16	25	20	18
Normal	24	21	13	23	8	6	23	13	15	24	18	12
Affected	1	0	0	0	0	1	0	1	1	1	2	6
Lost	0	0	0	0	0	0	0	0	0	0	0	0
TWO RP/BR SMOKE EXPOSURE DAYS												
Body posture (rest)	2	4	0	2	1	0	0	0	0	0	3	0
Body posture (exercise)	0	3	0	0	0	0	0	0	0	0	2	0
Respiratory congestion (rest)	0	0	3	0	0	0	0	1	1	0	0	3
Respiratory congestion (exercise)	0	1	0	0	0	0	0	4	0	0	0	7
Ungroomed coat	0	0	0	0	0	0	0	0	0	0	0	1
Aggressive responses	2	3	3	8	8	5	0	2	1	4	2	10
Vocalizations	16	9	3	21	17	13	22	9	5	28	23	15
Normal	16	7	3	21	15	11	22	7	4	28	13	12
Affected	0	2	0	0	2	2	0	2	1	0	6	3
Lost	0	0	0	0	0	0	0	0	0	0	4	0
THREE RP/BR SMOKE EXPOSURE DAYS												
Body posture (rest)										0	0	0
Body posture (exercise)										0	0	0
Respiratory congestion (rest)										0	3	3
Respiratory congestion (exercise)										0	9	5
Ungroomed coat										0	0	0
Aggressive responses										8	5	1
Vocalizations										9	10	7
Normal										9	10	4
Affected										0	0	3
Lost										0	0	0

(Continued)

RP/BR Target Concentration	0.0 mg/l			2.0 mg/l			4.0 mg/l			6.0 mg/l		
Successive 7-Session Blocks	Pre-[a]	Post 1[b]	Post 2[c]	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2	Pre-	Post 1	Post 2
FOUR RP/BR SMOKE EXPOSURE DAYS (REGULAR HANDLING)												
Body posture (rest)	0	0	0							0	0	0
Body posture (exercise)	0	0	0							0	1	1
Respiratory congestion (rest)	1	2	2							0	14	6
Respiratory congestion (exercise)	0	2	2							0	13	8
Ungroomed coat	0	0	0							0	0	0
Aggressive responses	9	11	10							1	4	2
Vocalizations	24	32	19							20	22	8
Normal	23	32	19							20	12	3
Affected	1	0	0							0	2	3
Lost	0	0	0							0	9	2
FOUR RP/BR SMOKE EXPOSURE DAYS (MINIMUM HANDLING)												
Body posture (rest)	1	0	0									
Body posture (exercise)	1	0	0									
Respiratory congestion (rest)	0	0	0									
Respiratory congestion (exercise)	0	0	1									
Ungroomed coat	0	0	1									
Aggressive responses	5	1	2									
Vocalizations	19	13	13									
Normal	19	13	13									
Affected	0	0	0									
Lost	0	0	0									

[a] Refers to a 7-day period prior to RP/BR-aerosol or filtered-air exposure--data are the total recorded counts for each symptom category for 6 prairie dogs during this period.

[b] Refers to the first 7-day period beginning on the day after the last RP/BR-aerosol or filtered-air exposure--data are the total counts for each symptom category for 6 prairie dogs during this period.

[c] Refers to the Day 10 through Day 28 period--data are taken every third day (i.e., Days 10, 13, 16, 19, 22, 25, and 28) and are the total recorded counts for each symptom category for 6 prairie dogs during this period.

Appendix F. NVSL Histopathology Reports for Prairie Dog (May 10,1988) and Rock Dove Specimens (December 21, 1988)

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

National Veterinary Services Laboratories
P.O. Box 844
Ames, Iowa 50010

Subject: Final Laboratory Report--Histopathology of Prairie Dogs

Date: May 10, 1988

To: Ray T. Sterner, Ph.D.
USDA, APHIS, ADC
Denver Wildlife Research Center
Building 16, P. O. Box 25266
Denver, CO 80225-0266

Enclosed is a summary chart of histological lesions found in tissues from the prairie dogs submitted to the National Veterinary Services Laboratories on July 28, and December 3, 1987. A key to abbreviations used on the chart is also enclosed.

A unique lesion was the presence of intracytoplasmic inclusions, within ganglionic cells of the larynx, associated with ganglioneutitis in several animals. These changes are suggestive of a viral infection, but no viral particles were seen in tissues examined by electron microscopy.

Histological findings on pigeon tissues will follow.

Arthur J. Davis, DVM

A. J. Davis, D.V.M.
Pathology Investigations Section
Pathobiology Laboratory

3 Enclosures

HISTOPATHOLOGY SUMMARY CHART--NVSL ACC. NO. 87-38152/87RA473

Prairie dog #	Sex	Date Collected	Lung	Liver	Trachea	Larynx	Other
2	F	07/07/87	+++	+++	-	-	-
12	F	07/07/87	++/IP	+	+	GN	-
18	F	07/07/87	PG	+/N	-	ND	-
51	M	07/07/87	+	+++	-	GN	S
56	M	07/07/87	+++	++	+	GN	-
66	M	07/07/87	+++	++	+	GN/IB	-
6	F	07/06/87	+++	+++	+	-	S
19	F	07/06/87	-	++/N	+	GN	-
21	F	07/06/87	+	+++	ND	-	-
54	M	07/06/87	+	++	ND	GN	-
58	M	07/06/87	+	++	+	GN/IB	-
68	M	07/06/87	+	++	-	-	-
1	F	07/06/87	+++	+++	+	GN	S
11	F	07/06/87	++	+/N	+	-	-
15	F	07/06/87	+/IP	+/N	+	-	-
67	M	07/06/87	++	++	-	GN	H-NT
69	M	07/06/37	+++	+++	-	GN	-
73	M	07/06/87	++/IP	++	-	GN	-
7	F	07/07/87	+	+++	+	GN	S
8	F	07/07/87	IP	+++	+	GN	-
16	F	07/07/87	-	+/N	-	-	-
22	F	07/07/87	++	+++	+	GN/IB	-
49	M	07/07/87	-/FBG	+++	-	GN	S
57	M	07/07/87	-/PBLH	++	+	GN	-
71	M	07/07/87	-	++	++	GN	-

Prairie dog #	Sex	Date Collected	Lung	Liver	Trachea	Larynx	Other
27	F	11/16/87	+++/PG	+++	ND	GN	-
28	F	11/16/87	++	+++	-	GN	-
30	F	11/16/87	++/PBLH	++	+	-	-
33	F	11/16/87	+	++	+	GN	-
34	F.	11/16/87	+/IP	++	ND	GN	-
35	F	11/16/87	++/PBLH	+++	+	GN/IB	-
75	M.	11/16/87	++	++	+	GN	-
77	M.	11/16/87	++	+++	+	GN	-
88	M	11/16/87	+/IP/PLG	+++	ND	-	-
96	M	11/16/87	-	+	+	GN	-
98	M	11/16/87	++	+++	ND	GN	S
100	M	11/16/87	++	++	ND	GN	-
32	F	11/17/87	++	++	+	GN/IB	S
36	F	11/17/87	++/PBLH	+++	+	GN	S
38	F	11/18/87	PG/IP	++	-	GN	S
39	F	11/18/87	+++/IP	++	ND	-	S
42	F	11/17/87	PG	++	ND	GN/IB	S
43	F	11/18/87	PG	++	-	GN	?
45	F	11/17/87	+/PBLH	++	+	-	S
74	M.	11/17/87	ND	ND	+	GN	-
81	M.	11/18/87	+	+++	-	GN	-
83	M.	11/18/87	+	++	ND	GN/IB	-
90	M.	11/18/87	-	+++	ND	-	S
97	M.	11/17/87	++	++	-	GN	S
02	M.	11/17/87	+	+++	+	GN	-
06	M	11/17/87	++	+++	ND	GN	S
10	F	07/10/87	++	+++	+	GN	-
17	F	07/10/87	++	++/N	-	-	-
25	F	07/10/87	-	+++	ND	GN	-
50	M	07/10/87	++/PBLH	+++	+	GN	-
52	M	07/10/87	+++	++	ND	GN	-
55	M	07/10/87	++	++	+	GN	-

Prairie dog #	Sex	Date Collected	Lung	Liver	Trachea	Larynx	Other
3	F	07/08/87	++/IP	+++	+	ND	S
5	F	07/08/87	++/IP	+++	+	ND	-
20	F	07/08/87	+	++	+	-	-
62	M	07/08/87	+++	+++	+	GN	S
64	M	07/08/87	+/PBLH	++	+	-	-
70	M	07/08/87	+++	+++	-	-	-
4	F	07/08/87	++	+++	+	GN	S
9	F	07/08/87	++	+++	-	GN	-
14	F	07/08/87	++	++	+	-	-
59	M	07/08/87	++	+++	+	GN	-
61	M	07/08/87	++	+++	+	GN/IB	-
65	M	07/08/87	+++	++	+	GN	-
13	F	07/10/87	+	+	-	GN	-
23	F	07/10/87	++	++	+	GN/IB	-
24	F	07/10/87	++/PBLH	+++	+	-	-
53	M	07/10/87	+	++	+	GN	S
60	M	07/10/87	++	+++	+	GN/IB	-
63	M	07/10/87	++/PBLH	++	-	GN	-
72	M	07/10/87	+++	++	-	GN	-

KEY TO HISTOPATHOLOGY SUMMARY CHART

Lung:

-	= No lesion seen
+	= Mild multifocal hemorrhage and necrosis
++	= Moderate multifocal hemorrhage and necrosis
+++	= Severe multifocal hemorrhage and necrosis
PBLH	= Peribronchial lymphoid hyperplasia
IP	= Interstitial pneumonia
FBG	= Foreign body granuloma
PG	= Pulmonary granulomas (unknown cause)
PLG	= Pulmonary lipogranuloma

Liver:

+	= Mild, diffuse, hepatocellular swelling and degeneration with cholestasis
++	= Moderate, diffuse, hepatocellular swelling and degeneration with cholestasis
+++	= Severe, diffuse, hepatocellular swelling and degeneration with cholestasis
N	= Minimal to mild, multifocal, hepatocyte necrosis

Trachea:

-	= No lesion
+	= Mild, multifocal, epithelial ulceration and necrosis
ND	= Specimen not examined

Larynx:

IB	= Intracytoplasmic inclusion bodies in ganglion cells
GN	= Lymphocytic ganglioneuritis
S	= Sarcocystosis in skeletal muscle
-	= No lesion seen
ND	= Specimen not examined

Other:

H-NT	= Hemorrhage in nasal turbinate

LABORATORY REPORT CONTINUED	ACCESSION NO. 87-38152/87RA473	DATE 05/02/88	PAGE 1 OF 1
DENVER WILDLIFE RESEARCH CENTER, DENVER, CO			

Electron Microscopy Results:

A section of hematoxylin-eosin stained prairie dog pharynx was reprocessed for electron microscopy. No viral particles are discernible within or associated with the cytoplasmic inclusions observed in the ganglion cells.

S. J. Jenkins

S. J. Jenkins, Biologist
Clinical Pathology Section
Pathobiology Laboratory

CONTINUED ON PAGE

DATA PROCESSING CODES

VS FORM 10-6A
(JAN 73)

USDA-APHIS

United States Department of Agriculture

Animal and Plant Health Inspection Service

Science and Technology

National Veterinary Services Laboratories
P.O. Box 844
Ames, Iowa 50010

Subject:
Final Laboratory Report--NVSL Acc. No. 88-08849/RA122

Date:
December 21, 1988

To:
Ray T. Sterner, Ph.D.
USDA, APHIS, ADC
Denver Wildlife Research Center
Building 16, P. O. Box 25266
Denver, CO 80225-0266

Enclosed is a summary chart of histological lesions found in tissues from the pigeons submitted to the National Veterinary Services Laboratories on December 3, 1987. A key to abbreviations used on the chart is also included.

A. L. Jenny, D.V.M., M.S.
Head, General Pathology and
Pathology Investigations Section

Enclosure

Histopathology Results (88RA122):

The following is a listing of lesions found in 41 pigeons exposed to red phosphorus/butyl-rubber smoke and six nonexposed controls.

CONTROL PIGEONS

Pigeon No.	Sex	Nasal Turbinates	Epiglottis	Larynx	Trachea	Lung	Liver
27	M	TNA	NSL	NSL	NSL	PC	PPI/C
32	F	TNA	NSL	NSL	NSL	H/PC	PPI
40	F	TNA	NSL	LF	NSL	H/PC	PPI
51	F	TNA	NSL	NSL	NSL	H	PPI
84	M	TNA	NSL	TNA	NSL	C/H	PPI
113	M	TNA	NSL	NSL	NSL	TNA	PPI

EXPOSED PIGEONS

Pigeon No.	Sex	Nasal Turbinates	Epiglottis	Larynx	Trachea	Lung	Liver
19	M	TNA	NSL	NSL	NSL	PC	PPI/C
31	F	TNA	TNA	NSL	NSL	H	PPI
39	F	TNA	NSL	NSL	NSL	TNA	PPD/N
43	F	TNA	NSL	NSL	NSL	H/PC	PPI/CS
72	M	TNA	TNA	NSL	NSL	H/PC	C/PPI
94	M	NSL	TNA	NSL	NSL	C/H	PPI
61	F	TNA	NSL	NSL	NSL	C	PPI
71	M	TNA	NSL	NSL	NSL	C/PC	CS/PPI
75	F	TNA	NSL	NSL	NSL	H	PPI
76	M	TNA	NSL	NSL	NSL	H	PPI
81	F	TNA	NSL	NSL	NSL	PVLI	PPI
96	M	TNA	NSL	NSL	NSL	NSL	PPD/N
36	M	NSL	NSL	NSL	NSL	H/PC	PPI
42	M	NSL	TNA	NSL	NSL	H/PC	PPD/N
46	F	TNA	NSL	NSL	NSL	PC	PPI

Pigeon No.	Sex	N. Turbinates	Epiglottis	Larynx	Trachea	Lung	Liver
65	M	TNA	NSL	A	LI/A	C/E/A	C/A
66	F	TNA	NSL	NSL	NSL	C	C
69	M	NSL	NSL	NSL	NSL	H	PPI
30	F	TNA	NSL	NSL	NSL	PC	TNA
60	M	TNA	NSL	NSL	NSL	H	PPI
62	M	TNA	NSL	LI	LI	C/E	C
68	M	TNA	NSL	NSL/A	NSL/A	C/A	C/A
82	M	TNA	NSL	NSL	H	C	CS/C
87	F	TNA	NSL	NSL	NSL	PVLI/PC	CS
38	M	TNA	TNA	NSL	NSL	H/PC	NSL
44	F	NSL	TNA	NSL	NSL	H/PC	PPI/CS
45	F	NSL	TNA	NSL	NSL	PC	PPI
54	M	TNA	NSL	NSL	NSL	C/E	C
56	F	TNA	NSL	NSL	NSL	H	PPI
120	M	TNA	NSL	NSL	NSL	C/E/A	C/E/A
20	M	TNA	NSL	NSL	NSL	PC	NSL
55	F	NSL	NSL	NSL	A	C/E	C
63	M	TNA	NSL	NSL/A	NSL/A	C/E/A	C/A
74	F	TNA	NSL	NSL	NSL	C/H/T	C/PPD/N
106	F	TNA	NSL	NSL	NSL	C/E	PPI
111	M	TNA	TNA	TNA	NSL	C/E/A	C
49	M	TNA	TNA	NSL	NSL	NSL	PPI
		Other Specimen--Heart--NSL					
50	M	TNA	NSL	NSL	NSL	H	PPI/PPD/N
77	F	TNA	NSL	NSL	NSL	C	C
88	M	TNA	TNA	TNA	TNA	TNA	TNA
103	~~F~~ M	TNA	NSL	NSL	F	C/A	C/A
107	F	TNA	NSL	NSL	NSL	NSL	PPI

TNA - Tissues not available for examination
LI - Lymphocytic inflammation
PVLI - Perivascular lymphocyte infiltration
C - Congestion
E - Edema
T - Thrombosis
H - Hemorrhage
PPI - Periportal inflammation
PPD - Periportal hepatocyte degeneration
N - Necrosis
CS - Cholestasis
PC - Pneumoconiosis
NSL - No significant lesion
LF - Lymphofollicular proliferation
A - Postmortem autolysis
F - Submucosal fibrosis

Comments and Summary

No significant lesions were found in nasal turbinates examined. Tissues from many pigeons were not available for microscopic examination.

No significant lesions were found in sections of epiglottis from control or exposed pigeons.

Lymphocytic inflammation was present in sections of larynx from one control and one exposed pigeon. This lesion is nonspecific.

No significant lesions were found in sections of trachea from control pigeons. Lesions found in sections of trachea from exposed pigeons included lymphocytic inflammation (2 pigeons), hemorrhage (1 pigeon), and fibrosis (1 pigeon). These lesions are considered to be nonspecific.

Hemorrhage was found in lung sections from four control and 15 exposed pigeons. This lesion may be related to the method of euthanasia. Pulmonary congestion and/or edema are presumed to be related to the method of euthanasia or hypostatic changes which occurred after euthanasia. Pneumoconiosis, a lesion found in three control and 12 exposed pigeons, is regarded as an incidental finding. Perivascular lymphocyte infiltrates were found in lung sections of two exposed pigeons. This lesion is considered to be nonspecific.

Lesions in the liver included periportal inflammation, a nonspecific lesion usually associated with a previous bacterial infection. Hepatic congestion is presumed to be related to method of euthanasia. Cholestasis was found in liver sections from five exposed pigeons. Cholestasis is a nonspecific finding. Periportal degeneration and necrosis was found in liver sections from five other exposed pigeons. This lesion was found in individuals from five different groups. The etiology of this lesion is unknown but does not appear to be related to level of exposure.

No lesions, attributable to red phosphorus/butyl-rubber smoke exposure, were found in the tissues examined.

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Appendix G. Graphic-planimeter Procedure for Estimation of Maximal Steady-state RP/BR Aerosol Concentrations.

A Lasico Planimeter (Model 120; Los Angeles Scientific Instrument Co., Los Angeles, CA) was used to measure the area under the curve of a 36 to 40 min portion of the ORNL Infrared Detector chart recordings at the maximal asympotic concentration levels. Areas under the chart recording curves of each entire exposure period (75 to 101 min) were also measured with this instrument. The ratio of this first to this second area for each given smoke exposure session was then multiplied by the total measured aerosol mass in order to estimate the fraction of total mass represented in the maximal-steady-state portion of each session. Since the gravimetric sampling rate for aerosol filter pads was 1.0 l/min, each resulting product was then divided by the number of min selected for maximal concentration measurements on each respective chart recording. These selected time intervals sometimes varied due to flame-outs and other aberrations. The resulting values are in mg/l units and they represent the best estimates for evaluating our achievement of "target concentrations."

The procedure has been validated using Task 1 data where actual maximal steady-state-phase concentration values were measured over 3, 10 min intervals for several RP/BR extrusion pump settings. A very high coefficient of determination value of 0.9769 was derived by correlating calculated planimeter concentration estimates to the actual measured amounts of RP/BR aerosol concentration using linear regression. Details of this validation data analysis are presented in Task 3 under this series of reports.

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